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Raised plasma levels of asymmetric dimethylarginine are associated with cardiovascular events, disease activity, and organ damage in patients with systemic lupus erythematosus

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ABSTRACT

Background. Asymmetric dimethylarginine (ADMA) is an endogenous nitric oxide inhibitor and a new independent risk factor for endothelial dysfunction and cardiovascular disease.

Objective. To investigate the relationship between plasma ADMA levels and cardiovascular events (CVEs) and disease characteristics in patients with systemic lupus erythematosus (SLE).

Methods. Demographic and clinical data were collected and plasma ADMA levels were measured in 107 patients with SLE. A modified organ damage index was calculated as defined by the Systemic Lupus International Collaborating Clinics/ American College of Rheumatology Damage Index (SDI), excluding CVE as an item.

Results. Cardiovascular disease, defined as ≥ 1 previous arterial CVE, was recorded in 16/107 (15%) patients with SLE and increased across tertiles of ADMA levels ($p = 0.023$ for trend). Mean plasma ADMA levels were significantly higher in patients with SLE with a history of CVEs than in patients without a CVE history ($p = 0.018$). In multiple regression analysis a high SLEDAI score, high modified SDI, high titre of anti-dsDNA antibodies, and low serum HDL were significantly associated with high plasma ADMA levels.

Conclusion. In patients with SLE, plasma ADMA levels are significantly associated with CVEs, measures of disease activity, and organ damage, independently of an unfavourable lipid profile.

INTRODUCTION

Cardiovascular disease, including coronary heart disease,[1] ischemic cerebrovascular disease[2] and peripheral vascular disease[3] has been recognized as an important cause of morbidity and mortality in patients with systemic lupus erythematosus (SLE). The mechanisms underlying the accelerated atherosclerosis in SLE are not completely clear because the traditional risk factors fail to account fully for the excess of cardiovascular events (CVEs) in lupus patients.[4]

Asymmetric dimethylarginine (ADMA) is an endogenous inhibitor of nitric oxide synthase[5] and is associated with endothelial dysfunction.[6] Furthermore, high ADMA plasma levels are a risk factor for acute coronary events[7] and a predictor of mortality and CVEs in patients with end-stage renal disease.[8]

In the presence of anti-dsDNA, up-regulation of methylation of arginine residues in proteins has been demonstrated in vitro.[9] As ADMA is released upon proteolysis of methylated proteins,[5] anti-dsDNA antibodies may be a trigger for enhanced ADMA production in SLE. However, this has not been studied in vivo.

The study aimed at assessing the hypothesis that plasma ADMA levels are associated with CVEs in patients with SLE, and with the presence of anti-dsDNA and other lupus characteristics.

PATIENTS AND METHODS

Data collection and clinical measures

One hundred and seven consecutive patients fulfilling the revised criteria for the classification of SLE were included. The local ethics committee approved the study. All patients provided informed consent. Demographic and clinical characteristics were systematically documented by questionnaire, chart review and clinical examination. Data collection comprised documented previous arterial CVEs. Coronary artery events were defined as myocardial infarction, coronary artery by-pass surgery, coronary angioplasty/stenting, and angina pectoris. Ischaemic cerebrovascular events were defined as transient ischaemic attacks or ischaemic stroke, or carotid endarterectomy. Peripheral artery events were defined as peripheral grafting or symptomatic peripheral artery ischaemia, confirmed by angiography.

Disease activity was measured by the SLE Disease Activity Index (SLEDAI) and European Consensus Lupus Activity Measure (ECLAM). A modified organ damage index was calculated as defined by the Systemic Lupus International Collaborating

Clinics/ American College of Rheumatology Damage Index (SDI), excluding CVEs as a damage item.

Biochemical measurements

ADMA was measured by high performance liquid chromatography, as published previously.[10] The upper limit of the reference range is 0.55 μmol/l. The subjects had fasted and had refrained from smoking and alcohol consumption for at least 24 hours before sampling. Laboratory investigations at the time of ADMA measurement included C-reactive protein, serum creatinine, immunological measures, and fasting levels of blood glucose, plasma homocysteine, serum total cholesterol, high density lipoprotein cholesterol and triglycerides.

Anti-dsDNA titres were evaluated using an indirect immune fluorescence technique with Crithidia luciliae as substrate. If the qualitative test in 1:10 dilution was positive, titres were measured.

Statistical analyses

ADMA levels in patients with SLE with and without a history of previous CVE were compared using the non-parametric (Mann-Whitney) test. Associations between ADMA levels and clinical and other biochemical variables were identified by univariate tests and subsequently by multiple regression analyses. To determine which variables were independently associated with ADMA levels, the variables with $p < 0.2$ in the univariate analyses and variables with supposed clinical relevance were used as potential independent variables in a stepwise multiple regression analysis with ADMA as dependent variable. Stability of the model was checked by tentatively adding to the (almost) final model single variables initially not included in the model, in order to check once more whether these variables could indeed be missed.

Statistical analysis was performed using SPSS 11.0 (SPSS Inc., Chicago, IL). A 2-sided value of $p < 0.05$ was considered significant.

Results

The characteristics of the 107 patients with SLE are shown in table 1. At least one previous arterial CVE was documented in 16/107 (15%) patients. Coronary artery events had occurred in seven (7%), ischaemic cerebrovascular events in 10 (9%) and peripheral artery disease in four (4%) of the patients.

Table 1. Demographic and clinical variables and potential risk factors for arterial cardiovascular disease*

Variables	All patients with SLE (n = 107)
Demogaphic variables	
Female sex, %	92
Age, years	41 ± 13
White, %	76
Clinical variables	
Disease duration, years	6.7 ± 6.7
SLEDAI	4.9 ± 4.1
ECLAM	3.1 ± 1.7
SDI (modified)	1.3 ± 1.7
Corticosteroid use ever, %	81
Current corticosteroid use, %	52
Duration of corticosteroid use, months	62 ± 69
Current daily prednisone dose, mg	13 ± 12
Hydroxychloroquine use ever, %	87
ESR, mm/1st hour	25 ± 24
CRP, mg/l	11 ± 16
Serum creatinine, μmol/l	88 ± 20
Creatinine clearance, ml/min	88 ± 26
Anti-dsDNA, IE/ml	34 ± 67
Potential risk factors for arterial cardiovascular disease	
BMI, kg/m2	25 ± 6
Current smoker, %	22
Ever smoker, %	54
Hypertension, %	31
Diabetes, %	7.5
Serum HDL cholesterol, mmol/l	1.44 ± 0.37
Serum LDL cholesterol, mmol/l	2.8 ± 1.03
Serum triglycerides, mmol/l	1.25 ± 0.65
Serum homocysteine, μmol/l	11.7 ± 4.4
Plasma ADMA, μmol/l	0.44 ± 0.08

*Except where indicated otherwise, values are the mean (SD). SLEDAI = Systemic Lupus Erythematosus Disease Activity Index; ECLAM = European Concensus Lupus Activity Measure; SDI = Systemic Lupus International Collaborating Clinics/American College of Rheumatology Damage Index; ESR = erythrocyte sedimentation rate, normal <10 mm/1st hour; CRP = C-reactive protein, normal <8 mg/l; serum creatinine, normal 60-110μmol/l; creatinine clearance, (140-age(years) x body weight (kg)/(serum creatinine (umol/l) x R), for men R = 0.86 and for women R = 1.01; BMI = body mass index; hypertension: defined by a physician diagnosis and/or treatment with anti-hypertensive drugs; diabetes: defined by a physician diagnosis and/or the use of anti-diabetic drugs; LDL cholesterol = total cholesterol - HDL cholesterol - (0.45 x triglycerides); ADMA = asymmetric dimethylarginine.

Association between plasma ADMA levels and previous CVE

The mean (SD) plasma ADMA level (0.48 (0.07) μmol/l) in patients with SLE with a history of CVE was significantly higher than in patients with SLE without a history of CVE (0.44 (0.09) μmol/l, p = 0.018). Figure 1 shows that the percentage of patients with SLE with previous CVEs increased across the tertiles of plasma ADMA levels (p = 0.023 for trend). Traditional risk factors for arterial cardiovascular disease as well as ADMA levels were not significantly associated with previous CVEs in multiple regression analyses (data not shown).

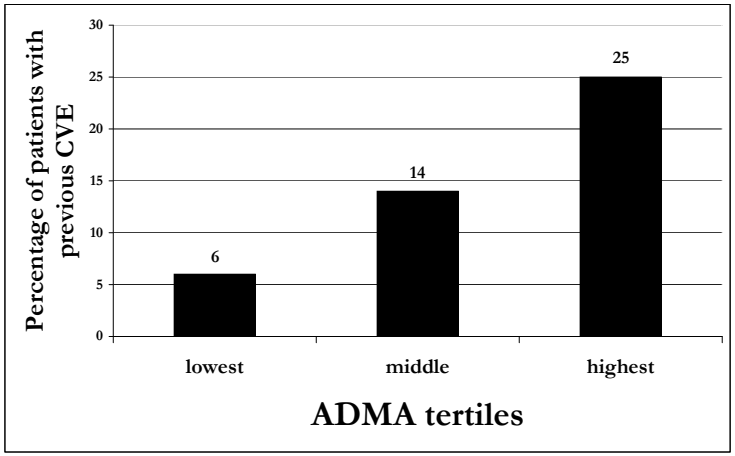

Figure 1. The percentage of patients with SLE with previous arterial CVEs increases across the tertiles of plasma ADMA levels (p = 0.023 for trend). In the lowest tertile (plasma ADMA levels ≤ 0.41 μmol/l) 2/36 (6%) patients had had a previous CVE; in the middle tertile (plasma ADMA levels range 0.42 – 0.47 μmol/l) 5/35 (14%) and in the highest tertile (plasma ADMA levels > 0.47 μmol/l) 9/36 (25%) had had a previous CVE.

Variables associated with plasma ADMA levels

Table 2 shows the results of univariate analyses.

In a stepwise multiple regression analysis a high SLEDAI score, high modified SDI, high titre of anti-dsDNA antibodies, and low serum HDL were significantly and independently associated with plasma ADMA levels (Table 3). The Pearson correlation coefficient between the SLEDAI score and ADMA levels was 0.503 (p < 0.01).

Table 2. Univariate analyses of variables possibly associated with plasma ADMA levels

Variables	B	SE	*p* value
CRP, mg/l	0.0017	0.0010	0.001
Serum HDL, mmol/l	-0.0670	0.0220	0.003
Serum triglycerides, mmol/l	0.0290	0.0130	0.027
Serum total cholesterol, mmol/l	0.0061	0.0070	0.381
Plasma homocysteine, μmol/l	0.0021	0.0020	0.284
Proteinuria	0.0910	0.0230	< 0.001
Creatinine clearance, ml/min	-0.0003	0.0001	0.323
Titre of anti-dsDNA, IE/ml	0.0006	0.0001	< 0.001
Complement C3, g/l	-0.0346	0.0280	0.226
Complement C4, g/l	-0.1620	0.1360	0.237
Lupus anticoagulant	0.0437	0.0200	0.032
IgM anticardiolipin	0.0002	0.0450	0.997
IgG anticardiolipin	0.0031	0.0210	0.885
SLEDAI	0.0108	0.0020	< 0.001
ECLAM	0.0218	0.0050	< 0.001
SDI (modified)	0.0125	0.0050	0.010
Age, years	0.0009	0.0010	0.140
Current smoking	-0.0037	0.0110	0.734
Diabetes mellitus	0.0411	0.0320	0.202

B = The regression coefficient; SE = standard error of B; CRP = C-reactive protein; SLEDAI = Systemic Lupus Erythematosus Disease Activity Index; ECLAM = European Consensus Lupus Activity Measure; SDI = Systemic Lupus International Collaborating Clinics/ American College of Rheumatology Damage Index.

Table 3. Multivariate analysis of variables associated with plasma ADMA levels

Variables	B	95% CI	Standardised B	*p* value
SLEDAI	0.0073	0.004 to 0.011	0.340	< 0.001
Titre of anti-dsDNA	0.0003	0.001 to 0.001	0.250	0.006
SLICC/ACR damage index (modified)	0.0097	0.002 to 0.018	0.190	0.019
serum HDL	-0.0004	-0.078 to -0.003	-0.171	0.037

CI = confidence interval. For other abbreviations, see table 2.

Discussion

The main finding of the present study is that high plasma ADMA levels were significantly associated with CVEs in patients with SLE. In addition, ADMA levels were significantly associated with measures of disease activity and organ damage. As far as we know, this is the first study of the association between ADMA levels and CVEs and disease characteristics in patients with SLE.

The increased mean ADMA level in the group of patients with SLE with a history of CVEs is in agreement with studies in other patient groups at high risk of the development of cardiovascular disease. Previous studies demonstrated increased oxidative stress in SLE[11] as well as raised plasma levels of circulating oxidized low density lipoprotein in patients with SLE with a history of CVEs.[12] The major route of ADMA elimination is degradation by the enzyme dimethylarginine-dimethylaminohydrolase (DDAH), which is very sensitive to oxidative stress.[13] Reduced DDAH activity by increased oxidative stress may thus contribute to increased ADMA levels in SLE.

The second important finding of our study is the association between ADMA levels and measures of disease activity, especially a high titre of anti-dsDNA antibodies. This observed association is in line with results of in vitro studies. Anti-dsDNA antibodies were shown to be reactive with the arginine-glycine-rich domains in recombinant heterogeneous nuclear ribonucleoprotein A2 (hnRNP A2).[9] Remarkably, these domains are also preferred sites for the methylation of arginine to ADMA by type 1 protein arginine methyltransferases (PRMT1).[14] In the presence of anti-dsDNA, methylation of hnRNP A2 by PRMT1 was increased to 3.5 times the control level. Therefore, anti-dsDNA antibodies may be a trigger for increased ADMA production by up-regulating methylation of arginine residues by PRMT1. Moreover, anti-dsDNA monoclonal antibodies enhance the inflammatory reaction by the release of proinflammatory cytokines from mononuclear cells.[15] These studies and our findings provide scientific rationale for the hypothesis that anti-dsDNA antibodies may have a role in the development of cardiovascular disease in SLE by enhancing ADMA production and by augmenting the inflammatory reaction.

Limitations of our study include the relatively small study group and the cross-sectional design. In our study, raised ADMA levels and traditional risk factors for CVEs were not independently associated with previous CVEs in multiple regression analysis. This finding might be explained by the relatively small study group and number of previous CVEs. Furthermore, cross-sectional data do not allow causality

to be established. A prospective study in a larger study group is required to answer definitively the question of whether raised ADMA levels are an independent risk factor for CVEs in patients with SLE.

The association between ADMA levels and modified SDI (excluding CVEs as an item) suggests that the nitric oxide pathway might also be involved in the development of damage in other organ systems in SLE. Further studies are advocated to elucidate the role of the nitric oxide pathway and its endogenous inhibitor ADMA in lupus pathogenesis and the development of organ damage in SLE.

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