CONCISE REPORT

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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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.

Cardiovascular disease, including coronary heart disease,[1] ischaemic cerebrovascular disease,[2] and peripheral vascular disease[3] has been recognised 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.

This 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.

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, 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

Abbreviations: ADMA, asymmetric dimethylarginine; CVE, cardiovascular event; DDAH, dimethylarginine-dimethylaminohydrolase; hnRNP, heterogeneous nuclear ribonucleoprotein; PRMT, protein arginine methyltransferase; SDI, Systemic Lupus International Collaborating Clinics/American College of Rheumatology Damage Index; SLE, systemic lupus erythematosus; SLEDAI, SLE Disease Activity Index

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

Variables	All patients with SLE (n = 107)
Demographic 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 h)	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/m^2)	25 (6)
Current smoker (%)	22
Ever smoker (%)	54
Hypertension (%)	31
Diabetes (%)	8
Serum HDL cholesterol (mmol/l)	1.44 (0.37)
Serum LDL cholesterol (mmol/l)	2.8 (1.0)
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 Consensus Lupus Activity Measurement; SDI, Systemic Lupus International Collaborating Clinics/American College of Rheumatology Damage Index; ESR, erythrocyte sedimentation rate, normal <10 mm/1st h; CRP, C reactive protein, normal <8 mg/l; serum creatinine, normal 60–110 μmol/l; creatinine clearance, (140−age (years)) × body weight (kg)/(serum creatinine (μmol/l)×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×triglycerides); ADMA, asymmetric dimethylarginine.

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 CVEs 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. The 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 two sided value of $p<0.05$ was considered significant.

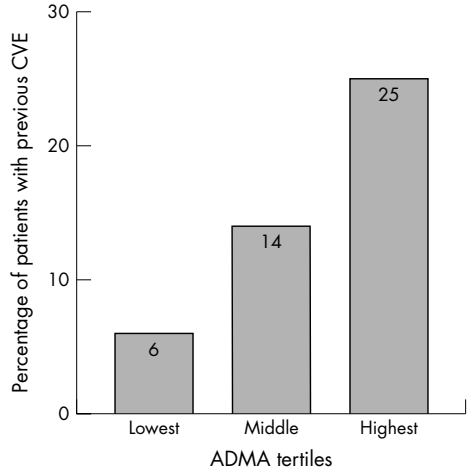

Figure 1 The percentage of patients with SLE with previous arterial CVEs increases across the tertiles of plasma ADMA levels ($p = 0.023$ for trend). In the lowest tertile (plasma ADMA levels ⩽0.41 μmol/l) 2/36 (6%) patients had had a previous CVE, in the middle tertile (plasma ADMA levels range 0.42–0.47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

RESULTS

Table 1 shows the characteristics of the 107 patients with SLE. 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.

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

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, regression coefficient; SE, standard error of B; CRP, C reactive protein; SLEDAI, Systemic Lupus Erythematosus Disease Activity Index; ECLAM, European Consensus Lupus Activity Measurement; 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 (IE/ml)	0.0003	0.0001 to 0.001	0.250	0.006
SDI (modified)	0.0097	0.002 to 0.018	0.190	0.019
Serum HDL (mmol/l)	−0.0004	−0.078 to −0.003	−0.171	0.037

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

(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).

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).

DISCUSSION

The main finding of this 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 oxidised 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 methyltransferase (PRMT1).[14] In the presence of anti-dsDNA, methylation of hnRNP A2 by PRMT1 was increased to 3.5 times that of 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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ECHO

Rituximab induces remission in stiff person syndrome

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The first report of successful treatment with a monoclonal anti-B cell antibody may offer new hope for patients with stiff person syndrome, a rare but ultimately fatal autoimmune disease of the CNS.

Monoclonal antibody specific for B cells expressing CD20 antigen (rituximab) alleviated severe symptoms when other treatments failed. It abolished intrathecal autoantibody against glutamic acid decarboxylase (GAD), suggesting that the syndrome is a B cell mediated autoimmune disease.

The 41 year old woman was an emergency admission in January 2004 with prolonged painful muscle spasms in her neck and back and arms and legs, rendering her bedridden and dependent on carers for months previously. She was taking baclofen and dantrolene sodium daily, fentanyl patches twice weekly and parenteral diazepam up to 80 mg and diamorphine up to 25 mg daily, providing subjective benefit.

The syndrome had been diagnosed in 2001. Various antispasmodic agents and disease modifying treatments, including seven courses of intravenous immunoglobulin and courses of cytotoxic drugs, tried since then had had no lasting success. Eventually, intrathecal infusions of hydrocortisone produced transient improvement, in December 2003.

However, after just over two weeks of rituximab at 375 mg/m^3, in January 2004, muscle spasms started to subside, and the woman was able to sit up and shower herself for the first time in two years. Testing in November 2003 showed intrathecal autoantibody to GAD, but at 17 days' treatment none was evident. One month after discharge her condition was stable and she needed only small doses of oral benzodiazepine and analgesia until symptoms recurred, at six weeks, when she was given further rituximab, with mycophenolate mofetil. She improved again after 14 days and was discharged, remaining well after five months.

Symptomatic treatment relies on γ-amino butyric acid (GABA) enhancing agents, but previous treatments modifying immune response by reducing antibody to GAD, a rate limiting enzyme in GABA synthesis, has had variable results.

▲ Baker MR, *et al*. *Journal of Neurology, Neurosurgery, and Psychiatry* 2005;**76**:999–1001.