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 who had 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, ischaemic cerebrovascular disease, and peripheral vascular disease 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.

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

In the presence of anti-dsDNA, up regulation of methylation of arginine residues in proteins has been demonstrated in vitro. As ADMA is released upon proteolysis of methylated proteins, 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 attack, ischaemic stroke, and 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. The upper limit of the reference range is 0.55 mmol/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 a control.

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

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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.

**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.
(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).

**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 as well as raised plasma levels of circulating oxidised low density lipoprotein in patients with SLE with a history of CVEs. The second important finding of our study is the association between ADMA levels and modified SDI damage in other organ systems in SLE. Further studies are 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. Furthermore, cross sectional data do not allow causality to be established. A prospective study in a larger study group is necessary to definitively answer the question of whether raised ADMA levels are associated with CVEs in patients with SLE.
ECHO

Rituximab induces remission in stiff person syndrome

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/m², 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.
