EXTENDED REPORT

Atherosclerosis and cardiovascular disease in systemic lupus erythematosus: effects of in vivo statin treatment

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ABSTRACT

Objective Statins may have beneficial vascular effects in systemic lupus erythematosus (SLE) beyond their cholesterol-lowering action, although the mechanisms involved are not completely understood. We investigated potential mechanisms involved in the efficacy of fluvastatin in preventing atherothrombosis in SLE.

Methods Eighty-five patients with SLE and 62 healthy donors were included in the study. Selected patients (n=27) received 20 mg/day fluvastatin for 1 month. Blood samples were obtained before the start and at the end of treatment. Monocytes from five patients were treated in vitro with fluvastatin.

Results Increased prothrombotic and inflammatory variables were found in patients with SLE. SLE monocytes displayed altered mitochondrial membrane potential and increased oxidative stress. Correlation and association analyses demonstrated a complex interplay among autoimmunity, oxidative stress, inflammation and increased risk of atherothrombosis in SLE. Fluvastatin treatment of patients for 1 month reduced the SLE Disease Activity Index and lipid levels, oxidative status and vascular inflammation. Array studies on monocytes demonstrated differential expression in 799 genes after fluvastatin treatment. Novel target genes and pathways modulated by fluvastatin were uncovered, including gene networks involved in cholesterol and lipid metabolism, inflammation, oxidative stress and mitochondrial activity. Electron microscopy analysis showed increased density volume of mitochondria in monocytes from fluvastatin-treated patients, who also displayed higher expression of genes involved in mitochondrial biogenesis. In vitro treatment of SLE monocytes confirmed the results obtained in the in vivo study.

Conclusions Our overall data suggest that fluvastatin improves the impairment of a redox-sensitive pathway involved in processes that collectively orchestrate the pathophysiology of atherothrombosis in SLE.

INTRODUCTION

Systemic lupus erythematosus (SLE) is associated with accelerated atherosclerosis and increased risk of cardiovascular disease (CVD),[1] which is not fully explained by traditional risk factors, suggesting that autoimmunity helps to accelerate atherosclerosis in a process that involves immune complex generation and changes in innate immune responses, complement activation, oxidative stress, increased production of adipokines, dysfunctional lipids, and changes in the production and activity of a complex network of cytokines.[2]

In addition to early-onset atherosclerosis, patients with SLE are also at risk of vascular damage, with vascular repair mechanisms being ineffective.[3] Antiphospholipid (aPL) antibodies (present in 20–50% of patients with SLE) have also been related to increased CVD, and a 'two-hit' hypothesis has been proposed in which circulating autoantibodies contribute to early endothelial cell (EC) dysfunction via interaction with β2-glycoprotein, although other thrombotic events are necessary to trigger plaque and clot formation.[4]

The inflammatory burden, along with excessive production of reactive oxygen species (ROS) and impaired antioxidant capacity,[5] may further drive the disease and cardiovascular complications. In fact, most atherosclerosis risk factors accelerate disease progression by augmenting oxidative stress. Yet, its precise role in SLE progression remains largely elusive.

Macrophages play important roles in the pathogenesis of atherosclerosis. Various parameters of circulating monocytes, including count, increased adhesive properties, lipid metabolism alterations, phagocytosis and low-density lipoprotein (LDL) cholesterol, are associated with CVD.[6 7] Therefore, treatments targeting monocytes–macrophages might effectively prevent cardiovascular events.

Statins inhibit cholesterol biosynthesis, and reduce cardiovascular morbidity, but they also have anti-inflammatory effects, including inhibition of inflammatory cytokine production, ROS formation and T-cell activation and upregulation of nitric oxide (NO) synthesis.[8] Statins also modulate inflammatory processes by reducing both the number and activity of inflammatory cells in atherosclerotic plaques. Favourable effects include modulation of cytokine secretion and signalling, decreased monocyte–EC adhesion, decreased expression of tissue factor (TF) and metalloproteases in macrophages, and inhibition of oxLDL-induced macrophage proliferation.[9 10]

We recently evaluated the effects of fluvastatin on the prothrombotic tendency of monocytes from

patients with antiphospholipid syndrome (APS), and showed multiple effects on monocyte activity, including the suppression of TF, protease-activated receptor (PAR) expression and macrophage proinflammatory activities, by inhibiting cytokines such as vascular endothelial growth factor and its receptor, fms-related tyrosine kinase 1 (VEGF/Flt1).[11] Although there is evidence of anti-inflammatory properties of statins, their mechanism of action and effects on SLE monocyte gene expression are unknown. The present study aimed to examine the anti-inflammatory effectiveness of fluvastatin in patients with SLE and to characterise the underlying mechanisms.

MATERIALS AND METHODS

Patients

Eighty-five patients fulfilling the classification criteria for SLE[12] and 62 healthy donors were included in the study (over a period of 24 months) after ethics committee approval had been obtained. All patients provided written informed consent. Exclusion criteria were: (a) severe disease activity needing aggressive treatment/treatment changes to induce remission; (b) any kind of infection within the 30 days before data collection; (c) immunobiological medication (infliximab, adalimumab, etanercept, rituximab, orabatacept) within the preceding 6 months; (d) coexistence of malignant lymphoproliferative disease; (e) HIV infection. Patients underwent a detailed clinical evaluation, with emphasis on SLE clinical manifestations, recurrent infections, current and previous medications, other autoimmune diseases, and determination of SLE Disease Activity Index (SLEDAI).[13] None of the healthy controls had a history of autoimmune disease, bleeding disorders, thrombosis or pregnancy loss. The characteristics of patients and controls are shown in table 1.

Twenty-seven stable and consecutive patients with SLE were given 20 mg/day fluvastatin for 1 month. These subjects were selected from patients with stable disease, without infections or changes in their treatment protocol at least 1 month before they started statin treatment, and they were not pregnant and not allergic or intolerant to statins. There were no statistically significant differences in demographic characteristics (age and sex) or in disease severity (data not shown). No treatment was given to healthy donors. Blood samples were obtained before the start and at the end of the treatment. During this treatment, patients were not withdrawn from their therapy. Clinical and laboratory variables of the patients included in the fluvastatin protocol are displayed in online supplementary table S1.

Blood samples

The collection of peripheral venous blood samples for obtaining plasma and serum and for purifying monocytes (Non-Monocytes Depleting Kit; Miltenyi Biotech, Bergisch Galdbach, Germany), lymphocytes and neutrophils (dextran sedimentation) was performed as described elsewhere[14–16]; for further details, see online supplementary methods.

In vitro studies

Monocytes isolated from five patients with SLE were treated with fluvastatin (20 μmol/L) (Sigma) for 19 h. Oxidative stress was measured by flow cytometry after 6 h of such treatment. Monocytes isolated from five healthy donors were treated for 17 h with either interferon (IFN)α (20 ng/mL; Inmunostep, Salamanca, Spain) or serum pooled from seven patients (25%).

Table 1 Clinical and laboratory characteristics of the patients with systemic lupus erythematosus and the controls

Characteristic	SLE patients (N=85)	Healthy donors (N=62)	p Value
Clinical			
Female/male	76/9	44/19	
Age (years)	39.47±12.40	36.69±10.47	NS
Anti-dsDNA	28.48±47.77	1.41±2.51	<0.001
aCL-IgG (GPL)	10.30±18.77	4.43±7.75	0.020
aCL-IgM (MPL)	13.99±46.44	10.47±6.55	NS
Anti-β2GPI (SGU)	2.84±5.59	5.04±7.87	NS
LA positivity	27 (31.8)	0	
SLEDAI	2.26±3.34		
Thrombosis	31 (36.5)	0	
Obesity	24 (28.2)	4 (6)	
Hypertension	14 (16.5)	0	
Diabetes	6 (7.1)	0	
Smoking	29 (34.1)	10 (16.1)	
Hyperlipidaemia	19 (22.4)	2 (3.2)	<0.0001
Nephropathy	24 (28.2)	0	
Increased CIMT	20 (23.5)	3 (5)	
Corticosteroids	57 (67.1)	0	
Antimalarials	59 (69.4)	0	
Laboratory*			
Total cholesterol (mg/dL)	187.42±34.01	197.46±35.67	NS
HDL cholesterol (mg/dL)	53.77±14.18	57.94±14.73	NS
LDL cholesterol (mg/dL)	114.08±28.04	121.33±30.41	NS
Triglycerides (mg/dL)	103.02±47.65	88.54±53.90	NS
C reactive protein (mg/dL)	3.68±6.36	1.21±1.20	0.001
Apolipoprotein A (g/L)	147.19±29.2	152.93±29.92	NS
Apolipoprotein B (g/L)	81.21±20.8	81.64±21.42	NS
C3 (mg/dL)	114.89±37.25	141.65±45.68	0.001
C4 (mg/dL)	18.84±8.59	28.15±11.81	<0.001

Values are number (%) or mean±SD.
aCL, anticardiolipin; CIMT, carotid intima–media thickness; dsDNA, double-stranded DNA; GPL, IgG phospholipid units; LA, lupus anticoagulant; MPL, IgM phospholipid units; SGU, standard IgG units; SLE, systemic lupus erythematosus; SLEDAI, SLE Disease Activity Index.

Flow cytometry analyses, analysis of oxidative stress biomarkers in white blood cells and plasma, western blotting and electrophoretic mobility shift assay

See online supplementary methods for details.

B-mode ultrasound carotid intima–media thickness (CIMT) measurements and thrombosis assessment

All patients and controls underwent B-mode ultrasound imaging for CIMT measurements. B-mode ultrasound imaging of the carotid arteries was performed as previously described[17 18] using Toshiba equipment (Aplio platform) with 7–10 MHz broadband linear array transducers. For further details, see online supplementary methods.

Electron microscopy

Electron microscopy (EM) analysis of monocytes isolated from patients with SLE before and after 1 month of fluvastatin treatment, or treated ex vivo with the drug, was performed as previously described.[19–21] Quantitative assessment of micrographs was carried out using stereology software (Wimasis, Cordoba, Spain). For further details, see online supplementary methods.

Microarray analysis and real-time quantitative PCR validation

Complementary RNA samples were prepared for hybridisation in an Agilent G4112F platform (Whole Human Genome Microarray 44k) using the One-Color gene expression system (for further details, see online supplementary methods). The raw microarray data were deposited in the Gene Expression Omnibus Database of the National Center for Biotechnology Information (accession no GSE45422).

Changes in selected genes were validated by quantitative real-time reverse transcription (RT)-PCR using the LightCycler thermal cycler system (Roche Diagnostics, Indianapolis, Indiana, USA), with glyceraldehyde-3-phosphate dehydrogenase as housekeeping gene, as described elsewhere.[14–16]

Statistical analysis

All data are expressed as mean±SD. Statistical analyses were performed with SSPS V.17.0. Following normality and equality of variance tests, comparisons were made by paired Student's t test or by a non-parametric test (Mann–Whitney rank sum test). Correlations were assessed by Pearson product–moment correlation, and association studies were performed using a χ^2 test. Differences were considered significant at $p<0.05$.

RESULTS

Prothrombotic and inflammatory variables are dysregulated in patients with SLE

Patients with SLE showed increased monocyte surface expression of TF and increased plasma levels of VEGF, interleukin (IL)-2, IL-6, IL-8, IL-17, IL-23, monocyte chemotactic protein-1 (MCP-1) and tissue plasminogen activator (tPA) compared with healthy controls (see online supplementary table S2). Almost a quarter (23.5%) of patients with SLE but only 5% of healthy donors showed increased CIMT; 36.5% of patients with SLE had previous thrombotic events (table 1).

Patients with SLE display a pro-oxidative status

Compared with healthy donors, monocytes and neutrophils from patients with SLE displayed increased peroxide and peroxynitrite production, mitochondrial membrane potential, and percentage of cells with altered $\Delta\psi_m$ (figures 1A–D). Total antioxidant capacity was found to be reduced in plasma from

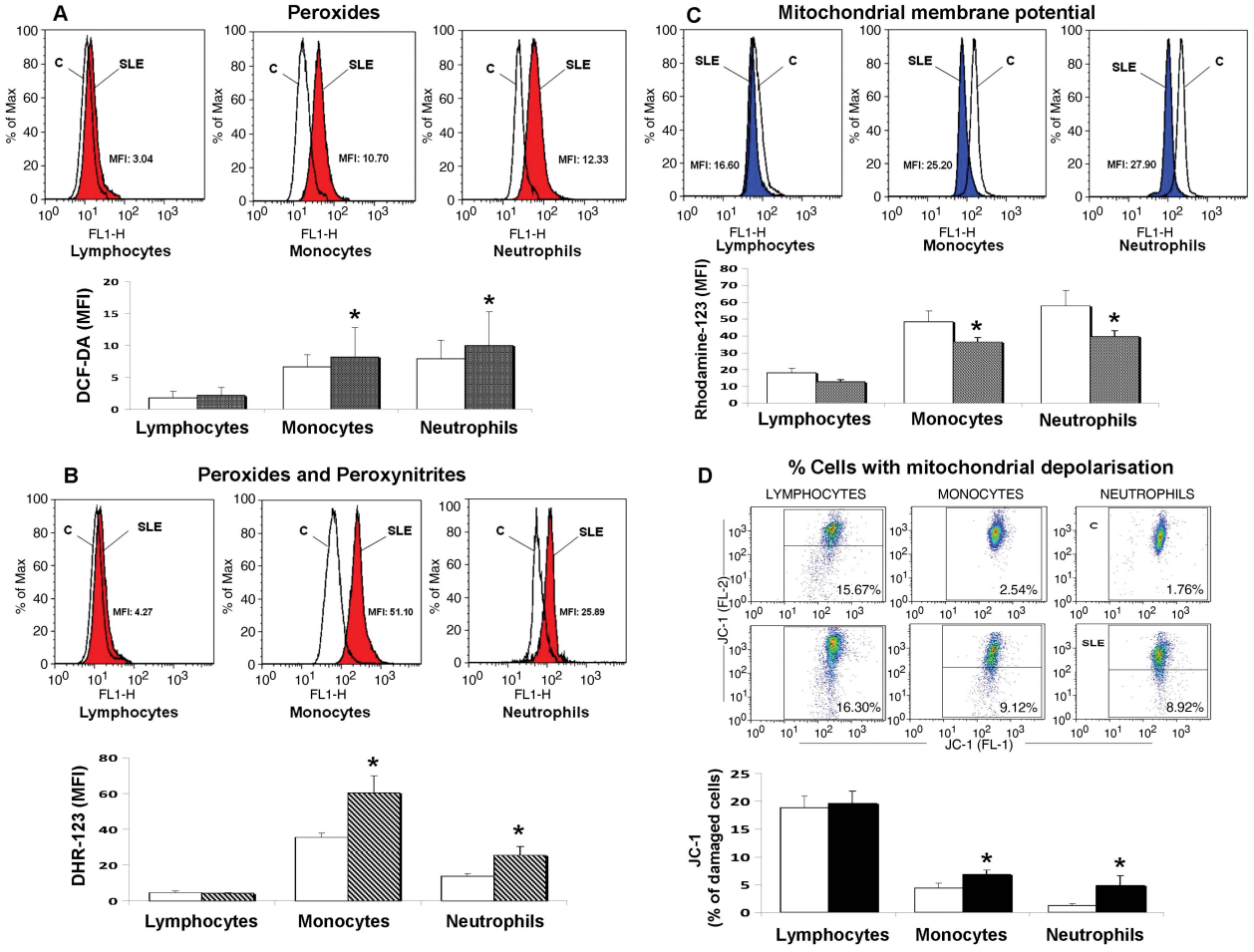

Figure 1 Cellular oxidative stress in patients with systemic lupus erythematosus (SLE). (A) Peroxide production in neutrophils, monocytes and lymphocytes of patients with SLE and healthy donors, determined by flow cytometry with the fluorescent probe DCF-DA. (B) Peroxide and peroxynitrite levels in neutrophils, monocytes and lymphocytes of patients with SLE and healthy donors, determined with the fluorescent probe DHR123. (C) Mitochondrial membrane potential in neutrophils, monocytes and lymphocytes of patients with SLE, determined with the fluorescent probe Rhodamine 123. (D) Proportion of circulating neutrophils, monocytes and lymphocytes with depolarised mitochondria, determined with the JC-1 MitoScreen assay. Representative histograms or dot plots are shown in parallel with bar graphs showing the mean±SD of mean fluorescence intensity (MFI) or percentage of damaged cells of all patients (dotted/solid bars) and healthy donors (empty bars) included in the study. FL1-H and FL2-H, fluorescence channels 1 and 2.

patients with SLE versus healthy donors (p=0.021), indicating a reduced ability to counteract ROS and resist oxidative damage (see online supplementary table S2). Also, plasma NO levels were significantly lower in patients with SLE versus healthy donors (p=0.005), probably as a consequence of its consumption after reacting with ROS forming peroxynitrites, as indicated by the augmented protein tyrosine nitration found in SLE monocytes (p=0.018). Mitochondrial superoxide dismutase (SOD) activity (p=0.021) was increased in patients with SLE versus healthy donors. Yet, catalase (CAT) and glutathione peroxidase (GPx) activities were notably reduced (p=0.043 and p=0.039, respectively) (see online supplementary table S2).

Correlation and association studies

Anti-double-stranded (ds)DNA antibodies correlated positively with some plasma inflammation markers (VEGF and MCP-1). A negative correlation was found between anti-dsDNA antibody levels and CAT in neutrophils from patients with SLE. Monocyte peroxide and peroxynitrite levels correlated positively with inflammatory markers such as VEGF and tPA, while GPx activity correlated negatively with plasma IL-6, IL-17A and IL-23 levels. A further positive correlation was demonstrated between the percentage of monocytes with depolarised mitochondria and inflammatory markers (MCP-1, macrophage inflammatory protein (MIP)1α and tPA) (see online supplementary figure S1).

Association studies (figure 2) indicated a relationship between CIMT and anticardiolipin (aCL)-IgG levels, age, cell surface TF expression in monocytes, plasma levels of MCP-1 and tPA, and oxidative status markers in monocytes and neutrophils, including DHR123 and GPx. The occurrence of thrombotic events in SLE was associated with factors related to autoimmunity (aCL-IgG and anti-dsDNA), inflammation (TF in monocytes, and plasma levels of MCP-1, IL-8, tPA and sP-selectin), and oxidative stress markers (DHR123 and the percentage of monocytes with altered $\Delta\psi_m$).

Monocytes are major players in atherothrombosis development in SLE

Many cytokines and inflammatory factors analysed in this study were altered in serum from patients with SLE and were related to disease status, CVD risk and early atherosclerosis. Notably,

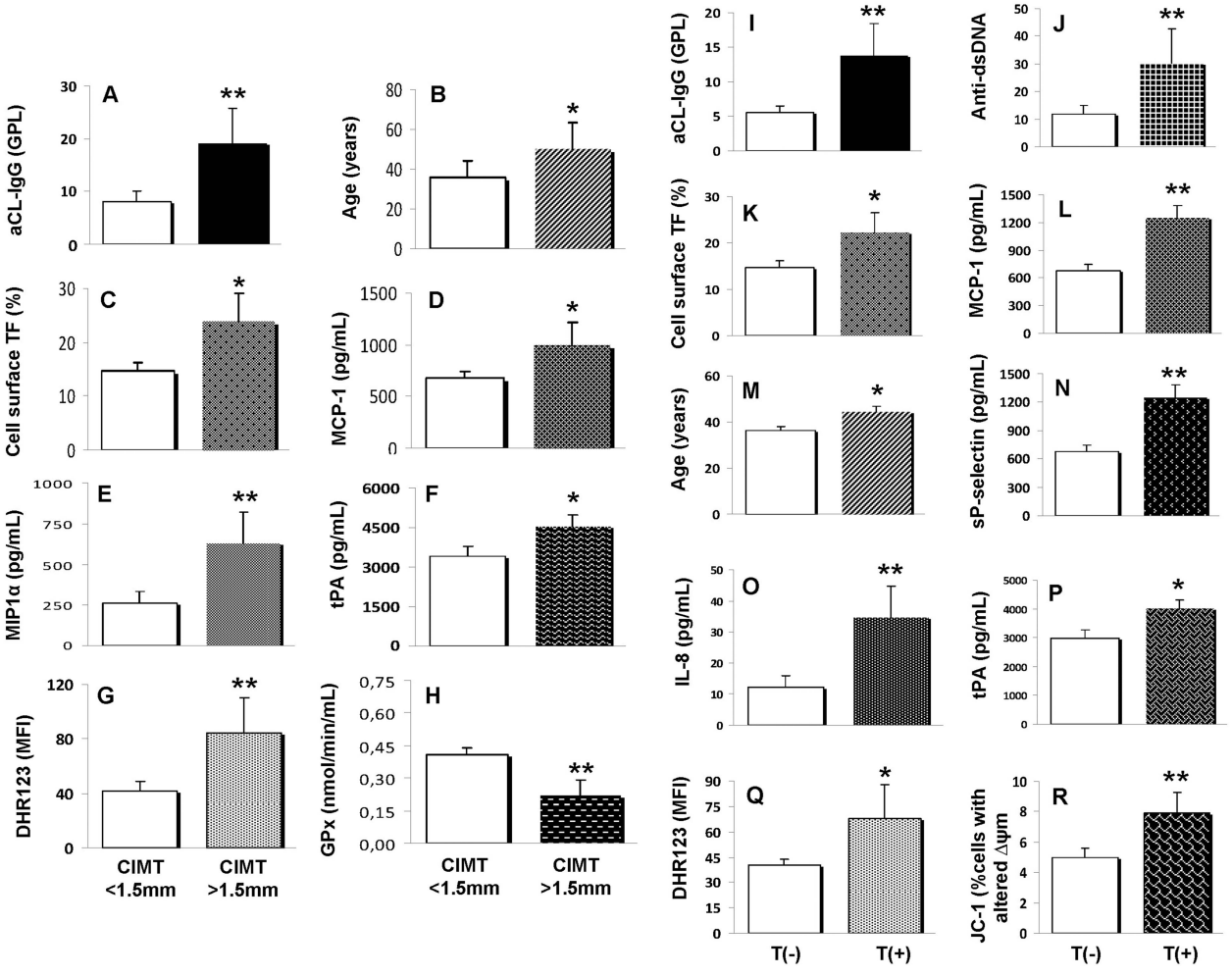

Figure 2 Association studies among inflammation, autoimmunity and oxidative stress markers, and increased carotid intima–media thickness (CIMT) or occurrence of thrombotic events (T) in systemic lupus erythematosus. (A–H) Relationship between the presence of increased CIMT and IgG isotype anticardiolipin antibody (aCL) levels, age, monocyte cell surface tissue factor (TF) expression, plasma levels of monocyte chemotactic protein (MCP)-1, macrophage inflammatory protein (MIP)1α and tissue plasminogen activator (tPA), monocyte peroxynitrite levels and monocyte glutathione peroxidase activity. (I–R) Relationship between the occurrence of thrombotic events (T) and IgG isotype aCL levels, anti-dsDNA antibody levels, monocyte cell surface TF expression, age, plasma levels of MCP-1, soluble P (sP)-selectin, interleukin (IL)-8 and tPA, monocyte peroxynitrite levels, and the percentage of cells with impaired $\Delta\psi$m (depolarised mitochondria). Significant difference versus patients without increased CIMT or versus patients without thrombosis (*p<0.05; **p<0.01).

gene expression analysis showed that monocytes from patients were major players in the altered proinflammatory variables mentioned above, while neutrophils and lymphocytes did not show increased mRNA expression of any of the cytokines/chemokines analysed, except MCP-1 (see online supplementary figure S2). The fact that SLE monocytes also showed altered oxidative status underlies the relevance of these cells in SLE pathophysiology. In support of this hypothesis, the in vitro treatment of normal monocytes with SLE serum or exogenous IFNα altered inflammatory and oxidative stress markers as well as mitochondrial membrane potential and biogenesis (see online supplementary figure S3). Effects were stronger in SLE serum-treated monocytes, particularly on thrombotic and inflammatory variables.

Fluvastatin reduced oxidative stress, serum chemokines/cytokines, and disease activity in patients with SLE

Treatment with fluvastatin for 1 month significantly reduced SLEDAI and anti-dsDNA levels in patients with SLE, while serum levels of some prothrombotic and proinflammatory variables (ie, IL-6, IL-8 and MCP-1) were also altered (see online supplementary table S3). Monocytes from fluvastatin-treated patients showed significant inhibition of TF protein expression and reversed activities of SOD2, CAT and GPx in relation to the untreated patients, along with decreased superoxide production and mitochondrial damage. Plasma NO and nitrotyrosine levels also returned to control values after fluvastatin administration.

Fluvastatin altered expression of genes involved in cholesterol and lipid metabolism, inflammation, oxidative stress and mitochondrial activity in monocytes

A total of 799 genes showed changes in expression compared with the control group using a twofold cut-off. In general, more downregulated (535) than upregulated (264) genes were found after statin treatment. Many novel target genes and pathways modulated by fluvastatin were uncovered.

Ingenuity Pathways Analysis (Ingenuity Pathways Analysis Knowledge Base; Ingenuity Systems) revealed a network of genes involved in cholesterol and lipid metabolism, inflammation, haematological disease and CVD, cell signalling, oxidative stress and mitochondrial activity (figure 3 and online

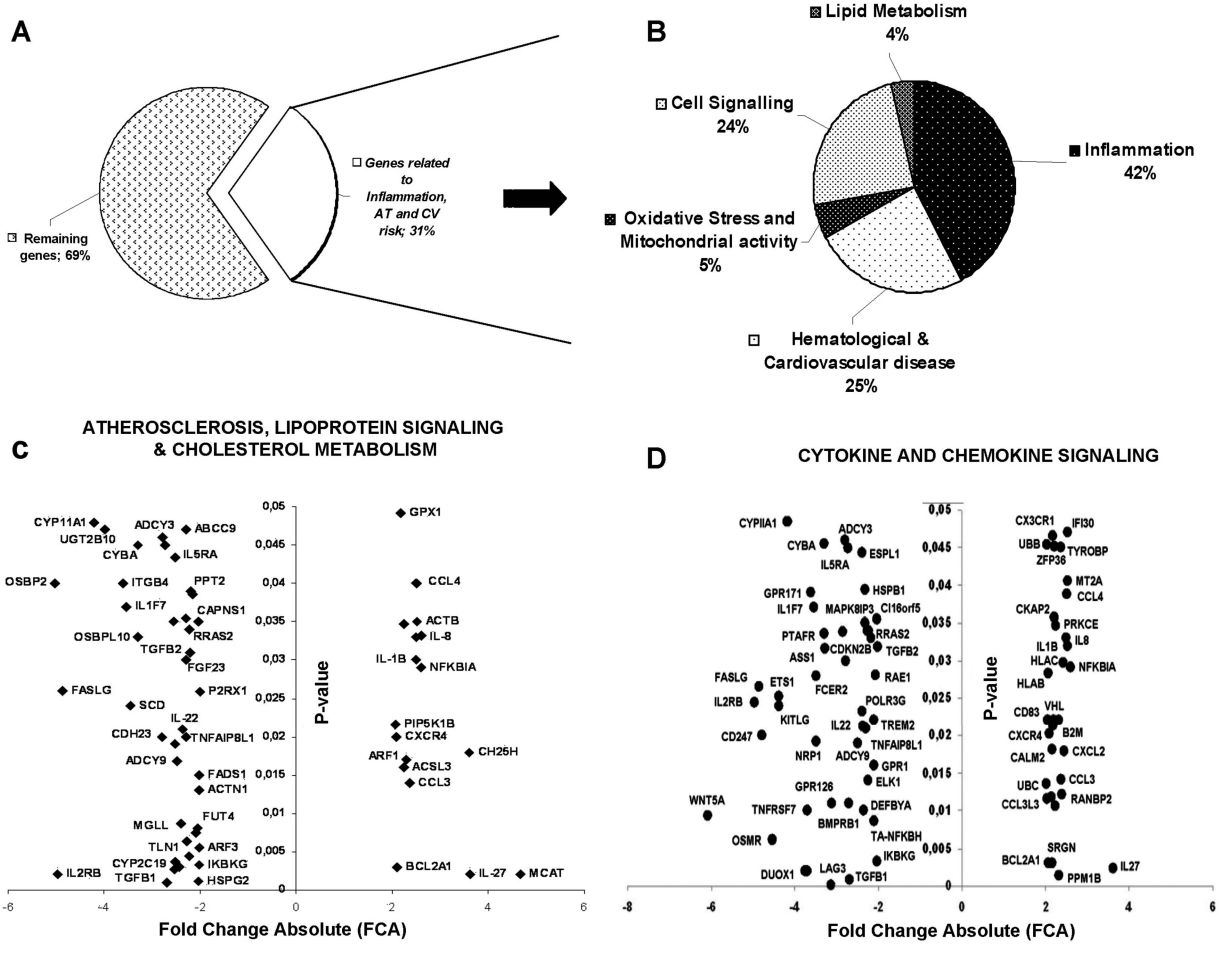

Figure 3 Fluvastatin alters gene expression in peripheral blood monocytes of patients with systemic lupus erythematosus (SLE). (A,B) Venn diagrams showing the functional categorisation of genes differentially expressed in patients with SLE after fluvastatin treatment in the area of atherosclerosis, inflammation and cardiovascular disease. Differentially expressed genes were classified and used for computational analysis to identify potential functional pathways and networks using the Ingenuity Pathways Analysis Knowledge Base (Ingenuity Systems). (C) Volcano plot between fold change absolute versus p value for genes related to atherosclerosis, lipoprotein signalling and cholesterol metabolism in fluvastatin-treated patients with SLE. (D) Volcano plot between fold change absolute versus p value for genes related to cytokine and chemokine signalling in fluvastatin-treated patients with SLE.

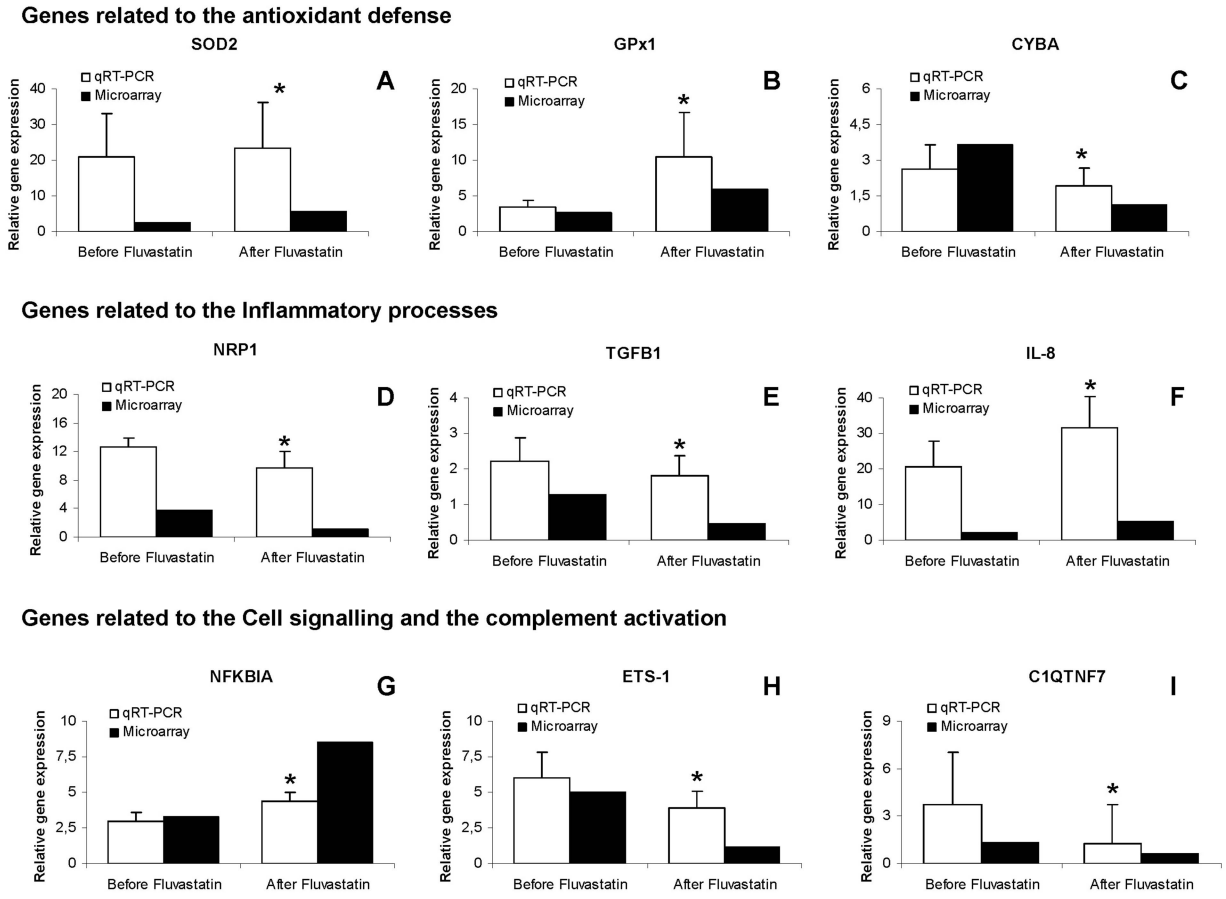

Figure 4 Microarray result validation by quantitative real-time reverse transcription-PCR (qRT-PCR). PCR was performed on selected genes belonging to the functional categories of antioxidant defence ((A) superoxide dismutase 2 (SOD2), (B) glutathione peroxidase 1 (GPx1), (C) cytochrome b-245 (CYBA)), inflammatory processes ((D) NRP1, (E) TGFβ1, (F) IL-8) and cell signalling and complement activation ((G) NFκBIA, (H) ETS-1, (I) C1QTNF7). PCR assays were consistent with the array results. *p<0.05 vs patients with systemic lupus erythematosus before fluvastatin treatment.

supplementary tables S4 and S5). Protein analyses further validated the data obtained (figure 4).

Fluvastatin promoted mitochondrial biogenesis in monocytes from patients with SLE

Our data indicate that fluvastatin prevented ROS production and regularised mitochondrial membrane potential in monocytes from patients with SLE (see online supplementary table S3). We next conducted an EM analysis and detected an increase in the number and volume density of mitochondria in monocytes from patients with SLE after fluvastatin treatment (figures 5A,B). Moreover, stereological analysis showed increased volume density of mitochondria after fluvastatin treatment (figure 5C).

In line with this observation, genes participating in mitochondrial biogenesis, including peroxisome proliferation-activated receptor α (PPARα), PPAR coactivator 1α (PGC-1α), nuclear respiratory factor (NRF)1, NRF2α, NRF2βγ and sirtuin 1 (SIRT1), were significantly increased in monocytes from fluvastatin-treated patients (figure 5D).

The in vitro treatment with fluvastatin of monocytes purified from patients with SLE reduced inflammatory and oxidative stress variables and induced gene changes similar to those observed in the microarray analysis (see online supplementary figure S5). Furthermore, mitochondrial biogenesis was also increased, as indicated by EM analysis and the increased expression of specific genes (see online supplementary figure S6). Thus, fluvastatin treatment increased monocyte mitochondrial mass, normalised mitochondrial membrane potential, and lowered ROS levels, potentially attenuating the damage caused by the disease.

DISCUSSION

This study covers important aspects of myeloid cell activity in patients with SLE, including activation, inflammation and oxidative stress in monocytes and neutrophils, which are master regulators of atherothrombosis in this autoimmune disease. Changes in the expression of a number of cytokines, chemokines, cell surface receptors, and EC regulators and markers related to autoimmunity and oxidative status in these patients were found to be intimately connected. Although these factors were compared with each other without statistical corrections or a prespecified or stratified set of hypotheses, our exploratory analysis allowed the creation of a framework for future research.

There is increasing evidence connecting an imbalance between various proinflammatory mediators with higher CVD risk.[1 22] Accordingly, inflammatory molecules (MCP-1, MIP1α, TF and tPA) were associated with premature atherosclerosis and/or the occurrence of thrombotic events in SLE in our series. However, the classical inflammatory cytokines (ie, IFNγ, tumour necrosis factor α, IL-1β, IL-6) that orchestrate common pathophysiological processes in SLE (ie, nephritis, skin manifestations, neurological affectations, etc) were not related to atherothrombotic events, suggesting molecular and cellular

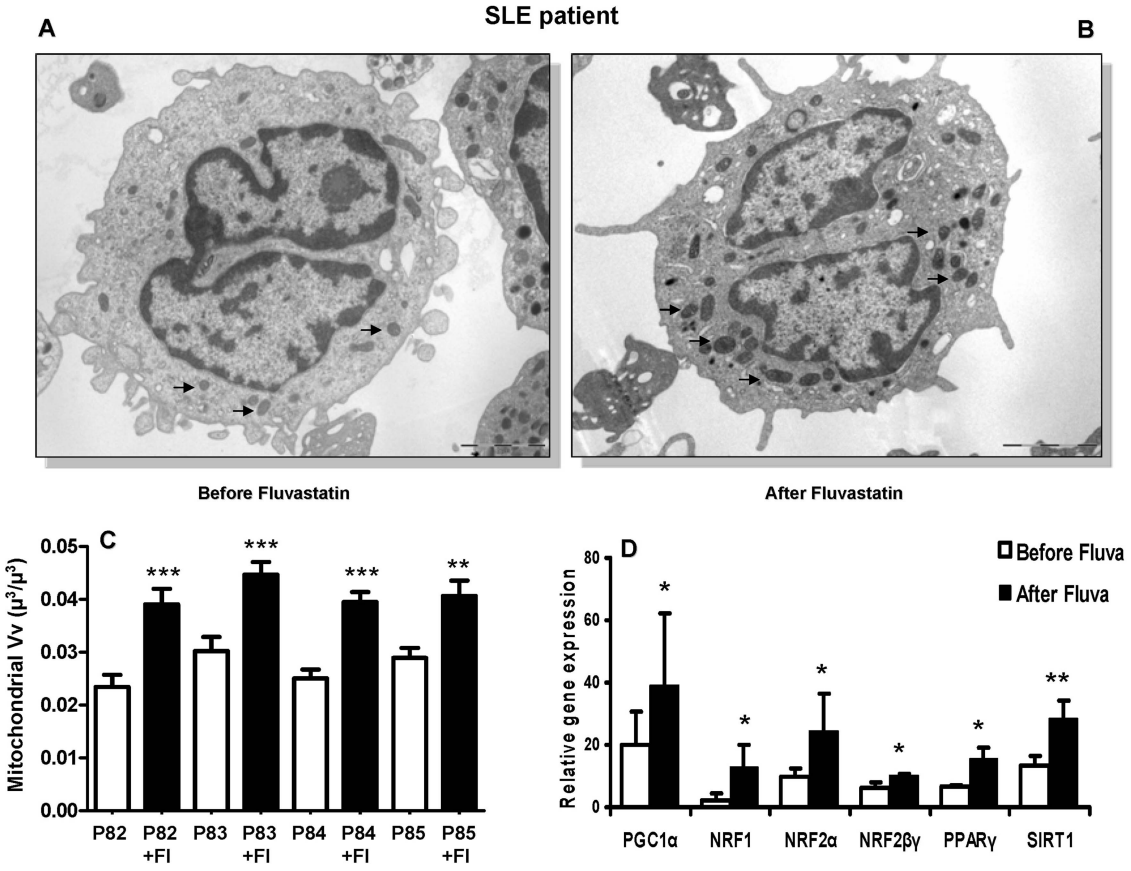

Figure 5 In vivo fluvastatin treatment promotes mitochondrial biogenesis in monocytes of patients with systemic lupus erythematosus (SLE). Representative electron microscope images of monocytes from a patient before (A) and after (B) fluvastatin treatment. (C) Stereological analysis showing volume density of mitochondria after fluvastatin treatment on four fluvastatin-treated patients. **p<0.01, ***p<0.001 versus patients before treatment. (D) mRNA levels of a set of genes involved in mitochondrial biogenesis. *p<0.05, **p<0.01 versus patients before treatment. NRF, nuclear respiratory factor; PPARα, peroxisome proliferation-activated receptor α; PGC-1α, PPAR coactivator 1α; SIRT1, sirtuin 1.

specificity of the cardiovascular comorbidity in these autoimmune patients. Nevertheless, because of the overlap in function, redundant activity and synergistic or antagonistic activity, the pathophysiological involvement of classical inflammatory cytokines cannot be ruled out.

In this study the use of purified peripheral blood subpopulations allowed evaluation of their inflammatory profile, highlighting the role of monocytes in SLE pathophysiology. Our results suggest that aberrant function of SLE monocytes plays a significant role in initiating and perpetuating the systemic autoimmune response, specifically the inflammatory damage.

Patients with SLE display marked abnormalities in neutrophil phenotype and function, with enhanced apoptosis and NETosis (cell death promoted by micro-organisms, proinflammatory cytokines and ROS) of neutrophils.[23] Evidence suggests that neutrophil dysfunction and increased NETosis might contribute to SLE pathology and vascular complications.[24] The results of our study (increased ROS production, mitochondrial dysfunction and MCP-1 overexpression) also show a significant role for neutrophils in the pathophysiology of SLE.

Additional risk factors involved in these processes were closely related to the characteristic autoimmunity of SLE. In particular, aCL-IgG antibodies were associated with the development and progression of SLE vascular inflammatory processes. Motoki and coworkers[25] recently showed that aPL antibodies contribute to arteriosclerosis in patients with SLE through TF induction and cytokine production by peripheral blood mononuclear cells.

Our findings confirm and extend previous reports on the existence of abnormal oxidative status profile in neutrophils from patients with SLE, demonstrating higher constitutive ROS production in monocytes and neutrophils compared with healthy donors.[26] Hyperactivity of oxidative metabolism in neutrophils and monocytes may participate in the ongoing subclinical immunological disorder observed in patients with apparently quiescent disease. This is in agreement with our correlation studies showing a direct relationship between oxidative stress markers, SLE autoantibodies (aCL-IgG and anti-dsDNA) and inflammatory proteins.

The increased production of ROS by monocytes promotes oxidative stress, leading to structural cell damage, including membrane lipids and mitochondria. Accordingly, mitochondrial perturbations were demonstrated in our series of patients, related to the inflammatory and prothrombotic status, as suggested by positive correlations with inflammatory markers and by the association between the increased percentage of monocytes with depolarised mitochondria and the occurrence of thrombotic events. It has been reported that mitochondrial dysfunction plays a role in inducing and maintaining inflammation and heart failure.[27] Also, we recently demonstrated the potential role of monocyte mitochondrial dysfunction in the development of thrombosis in APS,[28] extending the idea of the key mitochondrial involvement in the pathophysiology of both autoimmune diseases.

To date, only one study has evaluated in vivo the relationship between oxidative stress and vascular damage in patients with

SLE, reporting subclinical coronary microvascular dysfunction in the absence of traditional CVD risk factors, and associated with underlying inflammation (increased C reactive protein levels) and impairment of total antioxidant capacity of plasma.[29] Yet, our study is the first to demonstrate in vivo the relationship between autoimmunity, inflammation and oxidative stress variables in plasma and leucocytes from patients with SLE, as well as their association with atherosclerosis and vascular damage.

The in vitro treatment of monocytes with SLE serum or IFNα demonstrated significant alterations in inflammation and mitochondrial membrane potential/oxidative stress markers, supporting the relevance of the IFN signature in the pathogenesis of SLE, and pointing to monocytes as major players in atherothrombosis development in SLE.

Our gene expression array study shows that fluvastatin treatment has a global anti-inflammatory effect on macrophages, including attenuated expression of proinflammatory cytokines and regulated expression of molecules mediating lipid and cholesterol metabolism, as well as atherosclerosis and inflammatory signalling.

As expected, some proinflammatory mediators were downregulated by fluvastatin, while others exhibited increased expression. This might explain previous controversies about the effectiveness of statins in atherosclerosis and inflammation prevention in different cohorts of patients with SLE.[30–32] Therefore, a complex interaction of factors related to disease activity may also be involved in the response to the statin. It is also important to consider that some changes may not be exclusively attributed to the statin treatment, but instead to the combined effect of other drugs received by the patients, including immunosuppressive compounds or corticoids. Nevertheless, the overall response was improvement in disease status and reduction in proinflammatory and pro-oxidant status, demonstrated by parallel clinical and analytical follow-up studies. Thus, although confirmatory studies are warranted, treatment of SLE with fluvastatin may be worthwhile.

Gene expression array allowed us to better delineate the effects of fluvastatin on patients with SLE, enabling the identification of novel cytokines, chemokines and intracellular molecules involved in the response to the drug. The anti-inflammatory effect of fluvastatin can be at least partially explained by its effect on proinflammatory signal-transduction pathways. Several members of the nuclear factor (NF)κB signalling pathway displayed reduced expression levels, and various members of the signalling cascades leading to NFκB (cAMP/protein kinase A, RhoA signalling and oxidative stress signalling) showed altered expression. We and others have previously shown that statins inhibit the activity of NFκB and prevent the phosphorylation and degradation of the NFκB inhibitory protein, IκB, in the setting of APS.[11] Accordingly, fluvastatin downregulated mRNA levels of IκBKG, an activator of NFκB-inducing kinase,[33] and upregulated mRNA levels of NFκBIA, a potent inhibitor of NFκB nuclear import,[34] in monocytes from patients with SLE. Besides NFκB signalling, ETS1, a potent proinflammatory transcription factor mediating MCP-1 and phospholipase expression,[35] and the family of cAMP and Rho signalling pathways were downregulated by fluvastatin.

CVD prevention by statins is dependent not only on their lipid-lowering effects, but also on their beneficial effects on vascular redox signalling.[36] Accordingly, various markers related to oxidative stress in patients with SLE exhibited altered expression and/or activity in response to in vivo fluvastatin treatment. Moreover, gene arrays allowed the identification of previously unreported markers of oxidative stress and altered mitochondrial activity in response to fluvastatin treatment.

Mitochondrial studies of patients with SLE after fluvastatin treatment demonstrated the presence of more low-potential mitochondria in monocytes, with a lower ROS production. We also demonstrate that fluvastatin treatment of patients with SLE upregulated genes related to mitochondrial biogenesis, a highly regulated process operating through PGC-1α-dependent NRFs. The improvement in inflammatory and oxidative stress profile and the increase in mitochondrial biogenesis after in vitro treatment of purified monocytes from patients with fluvastatin suggest that alterations in SLE monocyte phenotype are probably a direct effect of fluvastatin, although complementary effects of global improvements in serology/disease activity cannot be ruled out.

Our results are congruent with the beneficial effects observed in patients with type 2 diabetes treated with thiazolidinediones or resveratrol, which stimulate mitochondrial biogenesis and reduce mitochondrial dysfunction.[37 38] Thus, the stimulation of mitochondrial biogenesis through pharmacological interventions shows great promise in attenuating the clinical phenotype associated with SLE.

CONCLUSIONS

Overall, our data suggest that: (i) several mediators of autoimmunity, inflammation and endothelial dysfunction orchestrate the pathophysiology of atherothrombosis in SLE; (ii) a redox-sensitive pathway in which mitochondrial alterations have a relevant role seems to elicit these pathological processes; (iii) fluvastatin has significant anti-inflammatory and antioxidative effects on SLE monocytes, which may partly explain the beneficial pleiotropic effects of statins on CVD in the setting of SLE.

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