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Research Article

Effects of Atorvastatin on Atherosclerosis and Atherogenesis in Systemic Lupus Erythematosus: A Pilot Study

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Objective. The effect of statins on atherogenesis in systemic lupus erythematosus (SLE) is poorly known. To inform a wider trial we performed a pilot study evaluating the intima-media thickness of the common carotid artery (CIMT) and some oxidative [beta-2-glycoprotein-1 complexed with oxidised low density lipoprotein (β_2GPIoxLDL)], metabolic [paraoxonase (PON), nitrate (NO_3^-), nitrite (NO_2^-) and nitrotyrosine (NT)], inflammatory [C-reactive protein (CRP) and serum amyloid A (SAA)], and lipid markers before and after 1 year of treatment with 40 mg of oral atorvastatin (AT). *Methods*. Randomised, double blind, placebo controlled pilot study on consecutive SLE patients: 17 SLE patients were randomised into the AT arm and 20 into the placebo arm. CIMT was measured by high-resolution sonography, PONa by a spectrophotometric method, NO_3^- and NO_2^- by a colorimetric assay and oxLDL-β_2GPI, NT, CRP, and SAA by Elisa. *Results*. After correction for age and disease duration oxLDL-β_2GPI decreased by 27% ($P = 0.002$) and PON/HDL ratio increased by 12% ($P = 0.01$) but CIMT did not change. *Conclusion*. This pilot study revealed a decrease of oxLDL-β_2GPI (oxidant marker) and an increase of PON/HDL ratio (antioxidant activity) after AT indicating a favourable effect of the drug on atherogenic pathways that should be explored on larger trials.

1. Introduction

Coronary artery disease (CAD) accounts for significant morbidity and mortality in patients with systemic lupus erythematosus (SLE) [1] being 8-fold more common than in the normal population after correction for the traditional Framingham risk factors [2]. First myocardial infarction occurs almost 20 years earlier than in the general population [3] with 90% of SLE women deceased between 16 to 37 years of age having severe atherosclerosis with greater than 75% occlusion in at least one coronary artery [4]. CAD explains the second curve of the bimodal mortality pattern in SLE, with early deaths attributed to the disease itself [5]. Because of the longer life expectancy of SLE early intervention is paramount to decrease the risk of vascular related death. Using high-resolution carotid ultrasound investigators have detected greater intima-media thickness of carotid arteries (CIMT) [6] and/or a greater frequency of carotid plaques in patients with SLE [7, 8] independently related to CAD [8]. Natural statins reduce cardiovascular risk and atherosclerosis progression [9] whereas synthetic statins such as atorvastatin (AT) induced a significant regression of CIMT in familial hypercholesterolaemia [10]. Accordingly we started a one-year placebo controlled pilot trial with AT to inform a larger study. The major endpoint was a change of the CIMT over a year and secondary endpoints were changes in some

atherogenic and inflammatory markers over the same time span.

2. Materials and Methods

2.1. Patients and Study Design. The study was designed as a pilot, randomised, double blind, and placebo controlled. SLE patients were stratified by age (age 40 or >40 years) and randomised in pharmacy by computer generated numbers in a 1 : 1 ratio to receive 40 mg of oral AT daily or placebo. A formal sample size calculation was not feasible as the main intention of this pilot study was to detect any change in CIMT that could inform a sample size calculation for a phase II trial. Between September 2006 and November 2008, consecutive SLE patients from the Leeds Connective Tissue Disease Clinic, who fulfilled the inclusion and exclusion criteria, were invited to participate in the study. Inclusion criteria were 4 or more American College of Rheumatology criteria for SLE, or 3 criteria with one lupus specific end-organ involvement [11], being 18 years of age and able to understand and provide informed consent and willingness to switch to AT if already on a different statin, unless contraindicated. Exclusion criteria were inability or unwillingness to give informed consent, acute or chronic infections, uncontrolled heart failure, uncontrolled endocrine disease, uncontrolled hypertension, malabsorption, acute liver disease, heavy alcohol intake, pregnancy or risk of pregnancy (not using appropriate contraception or planning pregnancy over the study period), breast feeding, previous statin intolerance, and previous statin induced myopathy. The study was carried out in accordance with the revised declaration of Helsinki and the hospital ethics committee granted ethical approval (reference MREC 04/012). For the purpose of the study participants were seen at baseline and after one year: at baseline case notes were reviewed with regards to traditional cardiovascular risk factors such as hypertension, lipid profile, tobacco use, personal and parental history of vascular occlusions (ischemic heart disease with or without myocardial infarction, ischaemic stroke, and peripheral vascular disease), and past and current medication. Participants underwent a physical examination and had their weight and blood pressure checked and women had a pregnancy test. At baseline and end of study routine blood samples were taken for full blood count, clotting screen including lupus anticoagulant, renal and liver function tests, lipid profile (triglycerides, total cholesterol, and high density and low density lipoprotein), glucose, antinuclear antibodies (ANA), double stranded DNA (dsDNA), IgG anticardiolipin antibodies (IgGaCL), and C_3 and C_4. At the same time points research blood samples were taken for beta$_2$-glycoprotein-1 complexed with oxidised low density lipoprotein (oxLDLβ_2GPI), C-reactive protein (CRP), serum amyloid A (SAA), nitrate (NO_3^-) and nitrite (NO_2^-), plasma nitrotyrosine (NT), and paraoxonase activity (PONa). The British Isles Lupus Assessment Group (BILAG) index, the SLE disease activity index (SLEDAI), and Systemic Lupus International Collaborating Clinics (SLICC) damage index were calculated at baseline and 12-month follow-up. We screened 86 consecutive SLE patients: 42 declined participation and 44 (51%) accepted. Of the latter, 7 were already on a different statin and were switched to AT: therefore 37 SLE patients were randomized: 20 in the placebo arm and 17 in the AT arm. After randomisation 7 patients from the placebo arm and 4 from the AT arm dropped out (29%): 2 moved away and 9 from both arms simply ceased to attend their scheduled visits. Final data were available for 17 patients in the AT arm of whom 12 were statin naïve and 5 were statin pretreated (simvastatin $n = 4$, pravastatin $n = 1$; switched to AT at study entry) and for 13 patients in the placebo arm.

2.2. Carotid Ultrasound Examination. At baseline and 12 months a Doppler ultrasound of the carotid arteries was performed with an ATL HDI 5000 sonograph equipped with a 5–10 MHz linear transducer. The CIMT was measured bilaterally at the common carotid artery, 1 cm distal from the carotid artery bifurcation, as this is the most reproducible and accurate area to assess [12]. The mean of the 3 readings taken on either side was then averaged for the purpose of statistical analysis.

2.3. High Sensitivity C-Reactive Protein, Serum Amyloid A, Nitrotyrosine, and oxLDLβ_2GPI Complex. Enzyme linked immune assays were employed to measure crude plasma nitrotyrosine (HyCult Biotechnology, Uden, The Netherlands), high sensitivity C-reactive protein (Biosupply Ltd, Bradford, UK), SAA (Europa Bioproducts, Ely, UK), and oxLDLβ_2GPI complex (Corgenix, Broomfield, Colorado, USA) and according to the manufacturer's instructions. CRP stands for the high-sensitivity test throughout the paper.

2.4. Nitrate and Nitrite. Nitrate (NO_3^-) and nitrite (NO_2^-) were determined using the Griess reaction, as previously reported [13]. Serum was diluted 1 : 4 with PBS (pH 7.4), and 200 μL of this solution was ultrafiltered by centrifugation at 10000 g for 1 h, using 10 kDa molecular weight filters (Ultrafree-MC, Millipore). Only clear and colourless filtrates were tested. The assay was performed in standard flat-bottomed 96-well polystyrene microtitre plates containing 50 μL/well of standard or sample. The assay was blanked against PBS. To each well were added 4 μL of nitrate reductase and 10 μL NADPH giving final concentrations of 6.3 U/L and 550 μmol/L, respectively. Plates were incubated at room temperature for 2 hours. NO concentration was then determined by the addition of 65 μL of Griess reagents 1 and 2 to each well except to blanks, and after 10 min incubation at room temperature the absorbance was read at 540 nm.

2.5. Paraoxonase Activity. Serum paraoxonase activity (PON) was measured according to Eckerson et al. [14] with minor modifications. Paraoxon (1 mM) (Sigma-Aldrich) freshly prepared in 50 mM glycine buffer containing 1 mM calcium chloride (pH 10.5) was incubated at 37°C with patients serum for 10 min in 96-well plates (PolySorp). Then *p*-nitrophenol formation was monitored at 412 nm. Enzyme activity was calculated with a molar extinction coefficient of 18.290 Mcm^{-1} and expressed as U/L, which is defined as 1 μmol of *p*-nitrophenol generated per minute per litre under

Table 1: Baseline demographic and clinical characteristics of participants.

	Placebo ($n = 14$)	AT naïve ($n = 15$)	AT continuing ($n = 5$)
Age (years), mean (SD)	45.9 (10.9)	47.6 (13.6)	54.8 (15.6)
Female *n* (%)	14/14 (100.0%)	15/15 (100.0%)	5/5 (100.0%)
Caucasian *n* (%)	11/14 (78.6%)	15/15 (100.0%)	5/5 (100.0%)
Black *n* (%)	3/14 (21.4%)	0/15 (0.0%)	0/5 (0.0%)
Disease duration, median (IQR)	15.00 (7.50 to 22.75)	6.00 (2.00 to 13.00)	10.00[#]
Systolic BP (mmHg), median (IQR)	131.00[*] (113.00 to 143.00)	120.00[^] (105.5 to 130.7)	135.00[#]
Diastolic BP (mmHg), median (IQR)	80.00[*] (76.50 to 81.50)	77.00[^] (64.00 to 82.25)	82.00[#]
IMT CC mm, median (IQR)	0.055[^] (0.050 to 0.064)	0.055[†] (0.043 to 0.064)	0.060[#]
Past medical history			
Diabetes, *n* (%)	0/14 (0.0%)	0/13 (0.0%)	1/5 (20.0%)
Asthma/chronic bronchitis, *n* (%)	1/14 (7.1%)	4/15 (26.7%)	0/5 (0.0%)
Renal disease, *n* (%)	1/14 (7.1%)	3/14 (21.4%)	2/4 (50.0%)
Hypertension, *n* (%)	8/14 (57.1%)	8/15 (53.3%)	5/5 (100.0%)
Ischaemic heart disease, *n* (%)	1/14 (7.1%)	1/14 (7.1%)	1/4 (25.0%)
Cerebrovascular disease, *n* (%)	2/13 (15.4%)	2/14 (14.3%)	2/4 (50.0%)
Peripheral vascular disease, *n* (%)	2/14 (14.3%)	5/14 (35.7%)	1/4 (25.0%)
Gut Disease, *n* (%)	1/14 (7.1%)	2/15 (13.3%)	1/4 (25.0%)
Hyperlipidaemia, *n* (%)	3/13 (23.1%)	3/12 (25.0%)	3/4 (75.0%)
Ever smoked, *n* (%)	8/14 (57.1%)	7/15 (46.7%)	2/4 (50.0%)
Pack years, median (IQR)	1.00 (0.75 to 28.75)	3.00 (1.00 to 19.00)	1[#] ($n = 1$)
BMI, median (IQR)	26.5[*] (23.5 to 29.2)	25.20 (22.58 to 32.46)	24.65[#]
Parental history, *n* (%)			
Diabetes mellitus	4/14 (28.6%)	3/13 (23.1%)	1/4 (25.0%)
Hypercholesterolaemia	3/13 (23.1%)	3/13 (23.1%)	1/3 (33.3%)
Myocardial infarction	6/14 (42.9%)	7/13 (53.8%)	1/4 (25.0%)
Myocardial infarction <50 yrs	0/14 (0.0%)	5/12 (41.7%)	0/4 (0.0%)
Ischaemic stroke	4/14 (28.6%)	5/13 (38.5%)	1/4 (25.0%)
Drug history, *n* (%)			
Prednisolone	7/14 (50.0%)	10/15 (66.7%)	3/5 (60.0%)
Hydroxychloroquine	7/14 (50.0%)	8/15 (53.3%)	2/5 (40.0%)
Methotrexate	2/14 (14.3%)	3/15 (20.0%)	0/5 (0.0%)
Antihypertensive	7/14 (50.0%)	6/15 (40.0%)	3/5 (60.0%)
Warfarin	1/14 (7.1%)	5/15 (33.3%)	0/5 (0.0%)
Aspirin	9/14 (64.3%)	2/15 (13.3%)	2/5 (40.0%)
Laboratory features			
IgG aCL >20 GPL, *n* (%)	1/14 (7.1%)	3/13 (23%)	0/5 (0.0%)
IgG aβ_2GPI >12 U/L, *n* (%)	2/14 (14.2%)	3/13 (23%)	0/0 (0.0%)
Lupus anticoagulant, *n* (%)	2/13 (14.2%)	3/15 (20%)	0/0 (0.0%)
Cholesterol, median (IQR)	5.15 (4.48 to 5.93)	4.30 (3.80 to 5.60)	5.00[#]
LDL, median (IQR)	2.80[*] (2.50 to 3.55)	2.70[^] (1.13 to 3.23)	2.30[#] ($n = 4$)
HDL, median (IQR)	1.75 (1.38 to 2.00)	1.35[^] (1.18 to 1.50)	2.60[#] ($n = 4$)
Creatinine, median (IQR)	79.00 (77.25 to 87.25)	82.00 (74.00 to 88.00)	88.00[#]
ESR, median (IQR)	15.0[*] (10.0 to 32.0)	16.00[*] (8.00 to 24.00)	20.00[#] ($n = 3$)

AT: atorvastatin; BP: blood pressure; BMI: body mass index; IQR: interquartile range; aCL: anticardiolipin; β_2GPI: beta-2-glycoprotein-1; ESR: erythrosedimentation rate. [#]Interquartile range not calculated due to small sample size [†]$n = 12$, [*]$n = 13$, and [^]$n = 14$.

assay condition. All laboratory staff was blinded with regard to the blood samples.

2.6. Statistical Analysis. Changes of CIMT and of measured variables at 1 year were analysed by ANCOVA, taking the change from baseline to 1 year as the dependent variable and defining treatment group as a fixed factor, with baseline values of each variable entered as covariates. The distributions of the residuals from each ANCOVA model were checked for normality; where severe departures from normality were

Table 2: Baseline disease activity of patients.

	Placebo ($n = 14$)	AT naïve ($n = 15$)	AT continuing ($n = 5$)
Organ involvement, n (%)			
BILAG max			
A	0 (0.0%)	0 (0.0%)	1 (20.0%)
B	5 (35.7%)	4 (26.7%)	0 (0.0%)
C	2 (14.3%)	3 (20.0%)	1 (20.0%)
D	7 (50.0%)	8 (53.3%)	3 (60.0%)
E	0 (0.0%)	0 (0.0%)	0 (0.0%)
n (%) C or above			
Constitutional	4 (28.6%)	0 (0.0%)	1 (20.0%)
Mucocutaneous	5 (35.7%)	3 (20.0%)	1 (20.0%)
Neurological	2 (14.3%)	3 (20.0%)	1 (20.0%)
Musculoskeletal	6 (42.9%)	5 (33.3%)	2 (40.0%)
Cardiorespiratory	1 (7.1%)	1 (6.7%)	0 (0.0%)
Gastrointestinal	1 (7.1%)	0 (0.0%)	0 (0.0%)
Ophthalmic	0 (0.0%)	0 (0.0%)	0 (0.0%)
Renal	0 (0.0%)	0 (0.0%)	0 (0.0%)
Haematological	1 (7.1%)	0 (0.0%)	0 (0.0%)
SLEDAI, median (IQR)	2.00 (0.00 to 6.50)	4.00 (0.00 to 6.00)	2.00[#]
SLICC total, median (IQR)	1.50 (0.75 to 3.00)	2.00 (0.00 to 3.00)	1.00[#]
n (%) > 0			
Ocular	2 (14.3%)	2 (13.3%)	0 (0.0)
Neurological	7 (50.0%)	8 (53.3%)	2 (40.0%)
Renal	1 (7.1%)	0 (0.0%)	1 (20.0%)
Pulmonary	2 (14.3%)	3 (20.0%)	0 (0.0%)
Cardiovascular	2 (14.3%)	1 (6.7%)	1 (20.0%)
Peripheral vascular	2 (14.3%)	1 (6.7%)	0 (0.0%)
Gastrointestinal	0 (0.0%)	0 (0.0%)	1 (20.0%)
Musculoskeletal	4 (28.5%)	4 (26.7%)	2 (40.0%)
Skin	0 (0.0%)	1 (6.7%)	0 (0.0%)
Gonadal failure	2 (14.3%)	1 (6.7%)	0 (0.0%)
Diabetes mellitus	0 (0.0%)	0 (0.0%)	1 (20.0%)
Malignancy	1 (7.1%)	1 (6.7%)	0 (0.0%)

AT: atorvastatin; BILAG: British Isles Lupus Assessment Group; SLEDAI: systemic lupus erythematosus disease activity index; SLICC: Systemic Lupus International Collaborative Clinics damage index; IQR: interquartile range. [#]IQR not calculated due to small sample size.

identified, the natural log of the variables was used instead. In this small pilot study statistical test results are presented as guidelines only, with corrections where stated.

3. Results

3.1. Changes in Measured Variables over 12 Months. In a preliminary analysis the statin naïve and the statin pretreated patients in the AT group showed substantive differences in some baseline values (Tables 1 and 2); therefore the 5 statin pretreated patients were removed from further analysis: this allowed the resulting AT and placebo groups to be evenly matched for body mass index and the maximum BILAG score, though there were still differences in age and disease duration. Therefore ANCOVA models of change in the various blood markers were created which included both age and disease duration as covariates, in addition to baseline values.

Posttreatment changes of oxLDLβ_2GPI (−27%) and CRP (−73%) as well as total cholesterol (−26%) and LDL cholesterol (−41.4%) were seen in the AT arm compared to the placebo arm. The same applied to PON (+17.1%) and the PON/HDL ratio (+10.5%). The direction of change for SAA was the same in the AT (−64%) and in the placebo group (−72%); the CIMT remained unchanged (Table 3).

After adjustment for age, disease duration, and baseline values the two groups differed only for the posttreatment changes of oxLDLβ_2GPI and PON/HDL (alongside total and LDL cholesterol) (Table 4).

4. Discussion

Patient accrual was modest in this study: almost 50% of patients deemed eligible to participate denied entry and of those who entered the study 29% dropped out after randomisation: with just 10 patients in the AT group

Table 3: Unadjusted summaries of measured variables pre- and posttreatment.

	Placebo ($n = 13$)			Atorvastatin ($n = 12$)		
	Pre	Post	Δ%	Pre	Post	Δ%
oxLDLβ_2GPI u/L						
Mean ± SD	2.6 ± 0.5	2.9 ± 0.5	11.5%	2.6 ± 0.7	1.9 ± 0.5	−26.9%
Median ± IQR	2.6 ± 0.7	2.8 ± 1.0	7.7%	2.8 ± 1.1	1.9 ± 0.7	−32.1%
CRP μg/mL						
Mean ± SD	3.0 ± 2.7	3.4 ± 2.3	13.3%	6.6 ± 8.4	1.9 ± 1.2	−71.2%
Median ± IQR	2.4 ± 2.7	2.8 ± 3.5	16.7%	2.9 ± 6.0	2.0 ± 2.0	−31.0%
SAA μg/mL						
Mean ± SD	13.5 ± 9.5	3.8 ± 2.4	−71.9%	14.5 ± 11.2	5.3 ± 4.8	−63.4%
Median ± IQR	16.2 ± 15.8	3.4 ± 4.9	−79.0%	9.9 ± 20.5	3.3 ± 5.2	−66.7%
NO_3^- μM						
Mean ± SD	31.6 ± 25.0	27.0 ± 16.9	−14.6%	20.2 ± 10.2	18.9 ± 9.6	−6.4%
Median ± IQR	21.8 ± 31.2	23.6 ± 24.9	8.3%	20.4 ± 19.0	17.6 ± 12.5	−13.7%
NO_2^- μM						
Mean ± SD	9.2 ± 4.9	9.6 ± 4.9	4.3%	13.1 ± 6.4	11.3 ± 4.2	−13.7%
Median ± IQR	6.6 ± 9.1	9.1 ± 4.4	37.9%	10.5 ± 10.0	10.5 ± 5.2	0.0%
NT Nm						
Mean ± SD	557.2 ± 1729.4	307.2 ± 673.1	−44.9%	266.6 ± 593.5	143.1 ± 177.1	−46.3%
Median ± IQR	15.9 ± 47.1	111.1 ± 151.8	598.7%	18.7 ± 84.0	78.5 ± 164.2	319.8%
TG mmol/L						
Mean ± SD	1.23 ± 0.61	1.17 ± 0.47	−4.9%	1.26 ± 0.78	1.07 ± 0.62	−15.1%
Median ± IQR	1.15 ± 1.08	1.10 ± 0.90	−4.3%	1.00 ± 1.08	0.95 ± 0.95	−5.0%
CHO mmol/L						
Mean ± SD	5.43 ± 0.96	5.50 ± 1.02	1.3%	4.36 ± 1.21	3.23 ± 0.62	−25.9%
Median ± IQR	5.15 ± 1.55	5.70 ± 1.35	10.7%	4.30 ± 1.70	3.35 ± 0.90	−22.1%
LDL mmol/L						
Mean ± SD	2.95 ± 0.67	3.16 ± 0.53	7.1%	2.55 ± 1.05	1.49 ± 0.57	−41.6%
Median ± IQR	2.80 ± 1.20	3.10 ± 1.00	10.7%	2.70 ± 1.10	1.40 ± 0.78	−48.1%
HDL mmol/L						
Mean ± SD	1.86 ± 0.58	1.81 ± 0.46	−2.7%	1.20 ± 0.27	1.28 ± 0.21	6.7%
Median ± IQR	1.75 ± 0.55	1.70 ± 0.65	−2.9%	1.25 ± 0.45	1.30 ± 0.35	4.0%
PON U/L						
Mean ± SD	101.36 ± 16.38	92.65 ± 23.18	−8.6%	78.31 ± 17.52	94.44 ± 18.40	20.6%
Median ± IQR	106.80 ± 13.22	103.38 ± 45.53	−3.2%	78.06 ± 30.03	104.52 ± 33.76	33.9%
PON/HDL						
Mean ± SD	58.29 ± 19.43	55.80 ± 16.21	−4.3%	70.92 ± 28.20	79.26 ± 21.46	11.8%
Median ± IQR	52.85 ± 29.09	59.46 ± 21.90	12.5%	62.88 ± 28.25	78.96 ± 32.08	25.6%
IMT CC mm						
Mean ± SD	0.058 ± 0.013	0.057 ± 0.014	−1.7%	0.057 ± 0.010	0.059 ± 0.010	3.5%
Median ± IQR	0.055 ± 0.018	0.050 ± 0.020	−9.1%	0.058 ± 0.015	0.060 ± 0.015	3.4%

Δ%: percentage difference; oxLDLβ_2GPI: oxidised low density lipoprotein-beta 2-glycoprotein-1 complex; CRP: C-reactive protein; SAA: serum amyloid A; NO_3^-: nitrate; NO_2^-: nitrite; NT: nitrotyrosine; TG: triglycerides; CHO: total cholesterol; LDL: low density lipoprotein; HDL: high density lipoprotein; PON: paraoxonase; IMT CC: intima media thickness of common carotid artery; SD: standard deviation; IQR: interquartile range.

providing data on CIMT changes our sample size fell just short of published rules of thumb which recommend between 12 [15] and 30 [12] patients per treatment group in a pilot study. After adjustment for baseline CIMT, age, and disease duration, there were very small posttreatment changes in CIMT in each arm. Two recent trials on SLE yielded negative results: in the first, 40 mg of AT daily over two years did not change the CIMT and coronary artery calcium [13] whereas 10 mg of rosuvastatin daily over two years was associated with a nonsignificant decrease in the CIMT [16]. With a different vascular measurement, 20 mg of AT daily over 2 months improved flow-mediated vasodilatation in SLE alongside significant decreases of total cholesterol, low density lipoprotein (LDL), and triglycerides [17]. Improvements

Table 4: Adjusted mean changes of measured variables (95% confidence interval).

Variable	Placebo ($n = 13$)	Atorvastatin ($n = 12$)	Difference	F	P
OxLDLβ_2GPI u/mL	0.29 (−0.06 to 0.64)	−0.62 (−0.99 to −0.26)	−0.92 (−1.45 to −0.39)	12.97	0.002
CRP μg/mL	−1.28 (−2.45 to −0.11)	−2.89 (−4.11 to −1.66)	−1.61 (−3.41 to 0.19)	3.47	0.077
SAA μg/mL	−10.28 (−12.26 to −8.30)	−8.53 (−10.60 to −6.46)	1.75 (−1.28 to 4.79)	1.45	0.243
NO_3^- μM	−0.62 (−8.00 to 6.76)	−5.58 (−13.30 to 2.14)	−4.96 (−16.27 to 6.35)	0.84	0.371
NO_2^- μM$^{\#}$	−0.09 (−0.34 to 0.15)[4]	0.10 (−0.14 to 0.35)	0.20 (−0.57 to 0.18)	1.20	0.288
NT nM$^{\#}$	0.95 (0.15 to 1.75)	0.65 (−0.18 to 1.49)	−0.30 (−0.93 to 1.52)	0.26	0.619
TG mmol/L	−0.02 (−0.35 to 0.30)[1]	−0.33 (−0.34 to −0.03)[2]	−0.31 (−0.77 to 0.15)	2.09	0.170
CHO mmol/L	0.38 (−0.19 to 0.96)[1]	−1.39 (−1.86 to −0.91)	−1.77 (−2.61 to −0.92)	19.64	<0.001
LDL mmol/L	0.42 (0.07 to 0.78)[2]	−1.13 (−1.47 to −0.79)[3]	−1.55 (−2.07 to −1.03)	40.23	<0.001
HDL mmol/L	0.04 (−0.10 to 0.17)[4]	−0.01 (−0.14 to 0.13)	−0.04 (−0.26 to 0.17)	0.18	0.677
PON U/L	1.15 (−14.48 to 16.79)[4]	3.40 (−12.24 to 19.03)	2.24 (−24.10 to 28.59)	0.03	0.860
PON/HDL	−7.19 (−17.45 to 3.07)[4]	13.04 (2.78 to 23.30)	20.23 (4.81 to 35.64)	7.54	0.013
PDN mg/day	1.00 (−1.07 to 3.07)[2]	−1.04 (−2.91 to 0.82)[4]	−2.04 (−5.00 to 0.91)	2.13	0.163
IMT CC mm	−0.002 (−0.006 to 0.002)	0.003 (−0.002 to 0.007)[2]	0.005 (−0.011 to 0.001)	2.59	0.125

oxLDLβ_2GPI: oxidised low density lipoprotein beta-2-glycoprotein-1 complex; CRP: C-reactive protein; SAA: serum amyloid A; NO_3^-: nitrate; NO_2^-: nitrite; NT: nitrotyrosine; TG: triglycerides; CHO: total cholesterol; LDL: low density lipoprotein; HDL: high density lipoprotein; PON: paraoxonase; PDN: prednisolone. Data adjusted for baseline age and disease duration. ([1]$n = 9$, [2]$n = 10$, [3]$n = 11$, [4]$n = 12$; $^{\#}$dependent variable = difference between ln (follow-up + 1) and ln (baseline + 1), covariate = ln (baseline + 1).

of total and LDL cholesterol have been consistent throughout different studies employing 10 mg of pravastatin daily for one month [18], 10 mg of rosuvastatin daily for three months [19] and for two years [16], and fluvastatin 10 mg over 7.3 years in SLE patients with renal transplants [20]. In agreement with these studies we noted similar trends for the total and LDL cholesterol. PON accounts for most of the protective effect of HDL against LDL oxidation hence against atherosclerosis itself [21] and its activity can be reduced in SLE [22]. To counteract excess LDL oxidation, beta-2-glycoprotein-1 (β_2GPI), the very target of antiphospholipid antibodies, binds to oxidised LDL (oxLDL) to form the covalent complex oxLDLβ_2GPI [22], plasma levels of which are increased in disorders characterised by enhanced lipid peroxidation: primary [23] and secondary antiphospholipid syndrome [24], SLE, chronic nephropathies, and diabetes mellitus [14, 25]. In this pilot trial AT treatment was associated with a significant increase in the PON/HDL ratio paralleled by a decrease in oxLDLβ_2GPI, testifying to a possible indirect "antioxidant" effect of the drug. Of the two inflammatory markers, SAA was unaltered while CRP showed a decreasing trend: in the two-year rosuvastatin trial CRP decreased by 30% [16] but in other interventional studies CRP remained unchanged, in [18, 19].

Owing to the loss of the biological activity of nitric oxide in the early phases of atherosclerosis [26] and given that oxidative metabolites of nitric oxide relate to disease activity in SLE [27, 28] we hypothesized a change after treatment between the two groups that did not occur.

5. Conclusion

Our pilot study revealed a poor accrual and a high number of dropouts suggesting that a multicentre trial rather than a single centre trial would be necessary to achieve the desired numbers. The posttreatment changes of the PON/HDL ratio and of oxLDLβ_2GPI are encouraging and have suitable plausibility to be employed as atherogenic markers in SLE to define the biological and clinical effects of different statins at varying doses. Pilot trial registration number is: MREC 04/012

Conflict of Interests

Dr. Luis Romulo Lopez is an employee of Corgenix Ltd. The authors declare that there is no conflict of interests regarding the publication of this paper.

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