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PAPER

Pioglitazone improves the cardiovascular profile in patients with uncomplicated systemic lupus erythematosus: a double-blind randomized clinical trial

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Objective: We studied the effect of pioglitazone on insulin levels, inflammation markers, high-density lipoprotein (HDL) composition and subclasses distribution, in young women with uncomplicated systemic lupus erythematosus (SLE).
Methods: This double-blind trial included 30 premenopausal women (30 ±8 years old) with SLE, who were randomized to pioglitazone (30 mg/day) or placebo treatment for 3 months. Plasma and HDL lipids were determined by colorimetric enzymatic assays, insulin by radio-immunometric assay, inflammation by immunonephelometry and HDL size and subclasses distribution by a native 4–30% polyacrylamide gradient gel electrophoresis.
Results: Compared with placebo, pioglitazone significantly increased HDL-cholesterol plasma levels (14.2%), reduced fasting insulin plasma levels (23.6%) and the homeostasis model assessment-insulin resistance (31.7%). C-reactive protein (70.9%) and serum amyloid A (34.9%) were also significantly reduced with the pioglitazone use, whereas the HDL particle size was increased (8.80 nm vs. 8.95 nm; $p = 0.044$) by changes in the distribution of HDL_{2b}, HDL_{3b}, and HDL_{3c} subclasses. The change in HDL size correlated with a rise in free and cholesterol–ester content in the HDL particles.
Conclusion: Pioglitazone significantly enhanced insulin sensitivity, reduced inflammation, and modified HDL characteristics, suggesting a potential beneficial effect of this drug in patients with SLE with a risk to develop cardiovascular disease.
Trial registration: This trial is registered at ClinicalTrials.gov Protocol Registration System, with the number NCT01322308. *Lupus* (2012) **21,** 27–35.

Key words: atherosclerosis; inflammation; insulin resistance; lipoproteins; systemic lupus erythematosus

Introduction

Systemic lupus erythematosus (SLE) is an autoimmune inflammatory disease in which accelerated atherosclerosis and its sequelae are recognized as one of the most frequent causes of morbidity and mortality in young women.[1] The etiology and pathogenesis of atherosclerosis in SLE are only partially explained by traditional risk factors.[1,2] Several studies have reported non-traditional markers of increased risk for atherosclerotic cardiovascular disease in patients with SLE, as higher prevalence of hyperinsulinemia and insulin resistance,[3,4] inflammation,[5] abnormal high-density lipoprotein (HDL) particle size distribution and

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composition,[6,7] as well as higher proportions of proinflammatory HDL particles.[8,9]

Insulin insensitivity is known to be associated with accelerated atherosclerosis and appears to be a risk marker for both myocardial infarction and stroke.[10–13] The impact of insulin resistance may involve proinflammatory disturbances due to dysfunctional insulin signaling in different tissues; in addition to the higher prevalence of insulin resistance in SLE patients, it is well known that autoimmune and chronic inflammatory disorders are associated with the development of accelerated cardiovascular disease.[14]

HDLs vary in composition, size, charge, and biological activities. It has been reported that some of these lipoprotein characteristics may be more important than the HDL plasma concentrations in predicting future coronary heart diseases (CHD).[15] Abnormal distribution of HDL subclasses, characterized by low large HDL levels and high small HDL plasma levels, has been reported in patients with SLE,[6,7] and in other insulin resistance states.[16,17] Moreover, it has been reported that in SLE patients, HDL particles are enriched in triglycerides and depleted of cholesterol-esters.[6] These abnormalities may result in HDL-attenuated oxidative activity,[18] reduced anti-inflammatory effect[19] and lower capacity to promote cholesterol efflux,[20] which is the first step in reverse cholesterol transport.

Pioglitazone is an insulin-sensitizing compound of the thiazolidinedione (TZD) type. TZD activates nuclear receptors called peroxisome proliferator-activated receptors (PPAR), which regulate the transcription of genes with key roles in the metabolism of carbohydrates and lipids as well as inflammation.[21–23] The qualitative effects of pioglitazone on HDL may also be important, because it has been shown to increase the mean HDL size and reduce the core triglyceride content relative to cholesterol-esters in HDLs from subjects with and without diabetes.[24–26]

Although it has been previously shown that pioglitazone reduces several cardiovascular risk factors and renal inflammation in a lupus-prone murine model,[14] there are no studies that have examined the effect of this drug on insulin plasma levels, inflammation markers and HDL composition together with the distribution of their subclasses in patients with SLE. Therefore, the objective of this work was to study the effect of treatment with pioglitazone upon these parameters in non-diabetic patients with SLE in clinical remission and without renal compromise.

Materials and methods

Patients

This was a prospective, randomized, double-blind, placebo-controlled, parallel group study conducted in Mexico City. Eligible participants were premenopausal women with SLE older than 18 years, attending the outpatient Rheumatology Clinic at three Mexico City community tertiary care hospitals; the National Institute of Cardiology "Ignacio Chávez", the number 1 Regional Hospital from the Mexican Institute of the Social Security and the Mexican General Hospital. All fulfilled the 1982 American College of Rheumatology criteria for the classification of SLE,[27] and to avoid the effect of other cardiovascular risk factors, we excluded patients with clinical evidence of menopause, diabetes, thyroid dysfunction, neurological, renal or liver disease, personal history of high blood pressure, CHD, cerebrovascular events, chronic or acute infections, malignancy, and use of drugs or alcohol abuse. At the time of the study, none of the patients was smoking, pregnant, breast-feeding, or taking hormonal or lipid-regulation drugs. None of them was positive to anticardiolipin antibodies, lupus anticoagulant or antibeta-2-glycoprotein-1. Subjects were included in the study after they signed the informed consent form for participation (Figure 1). The protocol was approved by the Institutional Ethics Committee of each hospital and was performed in accordance with the Declaration of Helsinki.

The study took place at the National Institute of Cardiology "Ignacio Chávez" from March 2007 to April 2010, where information about current and cumulative drug therapy or any disease was obtained through a questionnaire and physical examination applied to all participants. SLE disease activity was assessed using the Mexican modification of the Systemic Lupus Erythematosus Disease Activity Index (MEX-SLEDAI).[28] Based on the MEX-SLEDAI, at time of the evaluation patients were considered to have active disease when the score was higher than 5. Height (m), weight (kg) and blood pressure were measured, and body mass index calculated by dividing weight by height squared (kg/m^2).

Study protocol

The eligibility screening (visit 1) occurred 4 weeks before randomization. During this visit, qualified personnel provided dietary counseling on the American Heart Association weight-maintaining

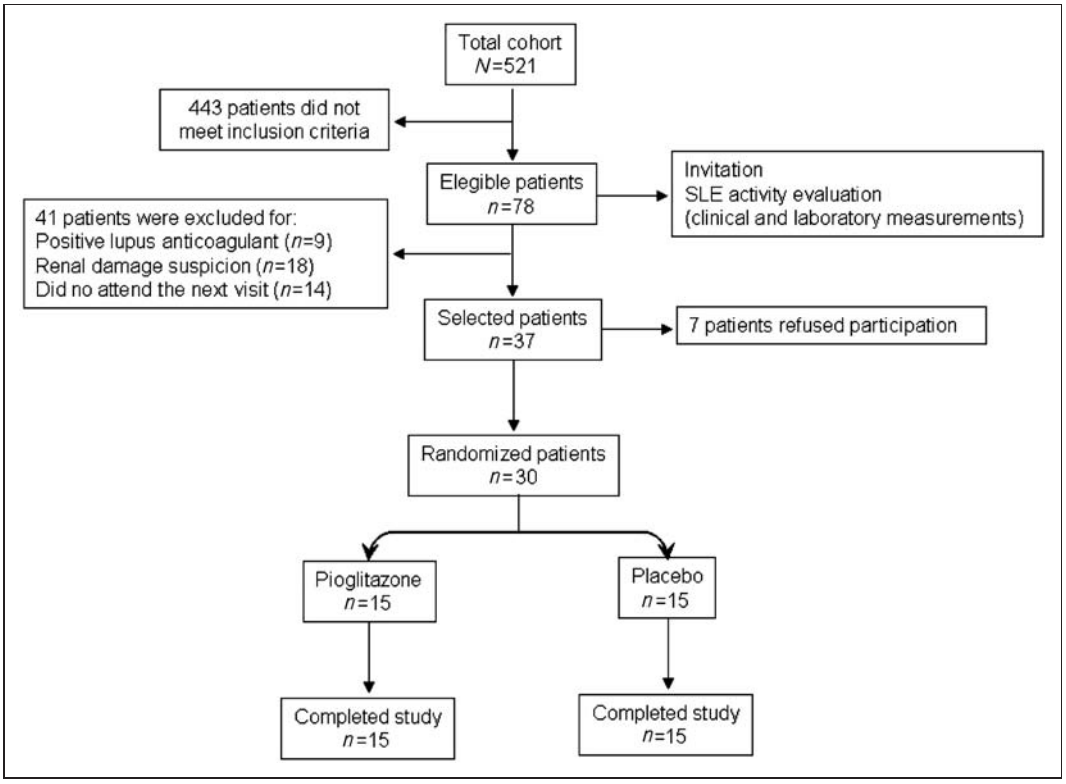

Figure 1 Flow chart for the selection and enrollment of patients.

Step 1 and selected patients instructed to follow this diet. At visit 2, selected patients were randomly assigned into two study arms; they had been informed, as part of the consent, that they would be receiving either a fixed dose of pioglitazone (30 mg) or placebo for 12 weeks once daily in the morning. Clinic visits occurred every 4 weeks from visit 3 to visit 5. At all visits, patients were received in the morning after fasting for at least 10 h. All patients use a reliable contraceptive method throughout the entire study. Pioglitazone and placebo were dispensed as pills similar in form and appearance. They were pre-packed in bottles and consecutively numbered. Each patient was assigned a treatment number and received the pills in the corresponding pre-packed bottle. An independent pharmacist dispensed the corresponding bottle according to a computer-generated randomization number list, and participants, care providers and researchers were blinded at treatment assignment. Compliance was assessed every clinic visit by tablet counting.

Laboratory methods

At visit 2 and visit 5, venous blood samples were obtained after a 12-h overnight fast. Ethylenedinitrilotetraacetate plasma was prepared by centrifugation at 4°C at 2500 rpm for 20 min and used for glucose, lipids, and lipoprotein measurements, or stored frozen at −70°C until their analysis. Aprotinin (100 KIU/ml) and benzamidine (1 mM) were used as protease inhibitors. Plasma glucose, total cholesterol, triglycerides, and HDL-cholesterol were measured using standardized enzymatic procedures in a Hitachi 902 analyzer (Hitachi LTD, Tokyo, Japan) and were considered within a normal range when their values were < 100 mg/dl, < 200 mg/dl, < 150 mg/dl and ≥ 50 mg/dl, respectively.[29] Accuracy and precision in our laboratory are under periodic surveillance by the Centers for Disease Control and Prevention Service (Atlanta, GA, USA). Low-density lipoprotein (LDL) cholesterol was estimated by using the Friedewald formula as modified by De Long et al.[30] Total high sensitive C-reactive protein (hsCRP), serum amyloid A (SAA), apolipoprotein B-100 (apo B-100) and apolipoprotein AI (apo AI) levels, were determined by immunonephelometry on a BN Pro Spec nephelometer (Dade Behring Marburg GmbH, Germany) according to the manufacturer's method. Interassay coefficients of variation were less than 3%. Plasma insulin concentrations were determined by

a radioimmunometric assay (Coat-A-count; Diagnostic Products, Los Angeles, California, USA). Insulin resistance was estimated with the use of the homeostasis model assessment-insulin resistance (HOMA-IR).[31] The normal values for insulin and HOMA-IR were $< 6.6\,\mu U/mL$ and < 1.3, respectively, according to the 75th percentile from a sample of healthy women, previously described.[6]

Distribution of HDL subclasses

HDL separated from plasma by ultracentrifugation at a density of 1.21 g/ml was loaded into a native 4–30% polyacrylamide gradient gel. After polyacrylamide gradient gel electrophoresis, gels were stained for proteins with Coomassie brilliant blue R-250, scanned and digitalized in a GS-670 Bio-Rad densitometer, using the software Molecular AnalystTM. Migration distance intervals of each gel was calculated by computing a standard curve of the protein-stainable high molecular weight standards (thyroglobulin, 17 nm; ferritin, 12.2 nm; catalase, 10.4 nm; lactate dehydrogenase, 8.2 nm; and albumin, 7.1 nm) as a function of their relative migration distance.[32,33] The relative proportion of each HDL subclass was estimated with the following size intervals: HDL_{3c} 7.21–7.76 nm; HDL_{3b} 7.76–8.17 nm; HDL_{3a} 8.17–8.77 nm; HDL_{2a} 8.77–9.71 nm; and HDL_{2b} 9.71–12.93 nm. The coefficient of variation for each subclass was less than 10%. The average HDL particle size represents the overall distribution of the HDL subclasses,[32,33] and was calculated as the average size of each HDL subclass interval (nm), multiplied by its relative area under the densitometric scan. Coefficient of variation for this determination was less than 1%.

Composition of HDL

Total protein, total cholesterol, free cholesterol, phospholipids, and triglycerides content of isolated HDL were determined in a Hitachi 902 analyzer, using commercially available enzymatic assays. Cholesteryl-esters were calculated by multiplying the difference between total and free cholesterol by 1.67.[34] Apolipoprotein content (apo A-I, apo A-IV, apo-E and apo-Cs) was evaluated semi-quantitatively by SDS–polyacrylamide gradient gel electrophoresis.[33]

Sample size calculation

Sample size calculation was employed to detect changes in HDL size using pioglitazone monotherapy.[25] We anticipated an increase in HDL size at 12 weeks from baseline, and calculated as $[(Z\alpha + Z\beta)\ SD/(\mu_0 - \mu_1)]^2 = [(1.65 + 0.84)\ 0.25\ /\ (8.4 - 8.6)]^2$.[25] Ten patients were needed in each group to have 80% power at the 5% significance level. We recruited and randomize 15 patients in each treatment arm to allow for dropouts.

Statistical analysis

All analyses were carried out using statistical software SPSS 13.0 for WINDOWS (SPSS inc., Chicago IL, USA). Results are expressed as mean ± standard deviation (SD) for normally distributed variables or median (interquartile range) for those with non-normal distribution. There were no outlier values in our study sample. Between-group differences were analyzed by Student's-*t*-test or Mann–Whitney *U* for independent groups, and within-group differences were analyzed by Student's *t*-test or Wilcoxon for paired samples. Spearman correlation coefficients were calculated to evaluate the relationship between all variables studied and the HDL size. All results with $p < 0.05$ were considered statistically significant.

Results

All 30 randomized patients followed and finished all study. The baseline clinical characteristics were similar in both groups (Table 1). For all studied women, the median SLE duration was 5.0 years (interquartile range: 2.0–10.0) and disease activity was 2.5 (1.2–4.7). Use of prednisone (46.7%) and antimalarials (83.3%) was similar in the two groups. Three patients (20%) in the placebo group and five (33%) in the pioglitazone group were taking more than 10 mg/day of prednisone, and two patients (13%) in each group were taking more than 200 mg/day of antimalarials. Patients of every group were clinically and biochemically inactive and no changes in SLE activity, pharmacology treatment, and blood pressure levels were observed during the study. No changes in the median of body mass index (24.9 vs. 24.8; $p =$ NS), hemoglobin (13.1 g/dl vs. 13.0 g/dl; $p =$ NS), hematocrit (39.1% vs. 38.4%; $p =$ NS), aspartate aminotransferase (21.3 U/l vs. 21.7 U/l; $p =$ NS) and alanine aminotransferase (16.1 U/l vs. 17.3 U/l; $p =$ NS) were observed after pioglitazone treatment. Similar results were observed in the placebo group (data not shown). According to pill count, all patients completed the study with an overall compliance of 94% (95% in placebo vs. 93% in pioglitazone group; $p =$ NS). Adverse effects were

Table 1 Baseline demographic and clinical parameters

	Total	*Placebo* (n = *15*)	*Pioglitazone* (n = *15*)	p^a
Age (years)	30 ± 8	29 ± 7	32 ± 10	NS.
Body mass index (kg/m^2)	24.7 (22.4–28.9)	24.5 (23.5–30.0)	24.9 (21.8–28.6)	NS
Waist circumference (cm^2)	81 (72–92)	81 (76–90)	82 (71–92)	NS
Systolic blood pressure (mmHg)	107 (97–118)	110 (90–120)	107 (98–115)	NS
Diastolic blood pressure (mmHg)	70 (65–76)	70 (60–80)	70 (65–75)	NS
SLE diagnostic (years)	5.0 (2.0–10.0)	4.0 (2.0–11.7)	5.0 (2.9–9.5)	NS
MEX–SLEDAI Index	2.5 (1.2–4.7)	4.0 (2.0–6.0)	2.0 (1.0–3.5)	NS
Hemoglobin (g/dl)	13.2 ± 1.1	13.1 ± 1.3	13.2 ± 0.9	NS
Leucocytes (µl)	4950 (4000–6050)	5000 (4000–5400)	4900 (3700–8600)	NS
Lymphocytes (µl)	1270 (1000–1550)	1100 (1000–1500)	1300 (1000–1700)	NS
Platelet count(×1000/µl)	237 (179–265)	236 (145–292)	238 (215–253)	NS
Erythrocyte sedimentation rate (mm/hr)	18.5 (14.2–31.0)	18.0 (14.5–27.0)	26.0 (12.0–32.0)	NS
Serum complement 4 (mg/dl)	14.1 ± 3.9	14.5 ± 3.9	13.7 ± 4.1	NS
Serum complement 3 (mg/dl)	94.8 ± 21.0	98.6 ± 22.3	91.1 ± 19.3	NS

Mean ± S.D. or median (interquartile range).
[a] = Student *t*-test for mean or Mann–Whitney *U* for median.
SLE, Systemic Lupus Erythematosus; MEX–SLEDAI, Mexican Modification of Systemic Lupus Erythematosus Disease Activity Index.

reported in three patients in the placebo group (headache, chest tightness, paresthesia, dizziness, nausea and joint pain) and in two patients in the pioglitazone group (headache and insomnia). All these adverse effects disappeared after the 4th week of treatment.

Lipids, glucose, and insulin parameters are shown in Table 2. Pioglitazone increased HDL-cholesterol levels (14.2%; $p = 0.029$) after 12 weeks of treatment. No changes were observed in the placebo group. Total and LDL cholesterol, triglycerides, apolipoproteins AI and B-100, as well as glucose levels were similar between both groups at baseline and after treatment. Fasting insulin levels and HOMA-IR were not statistically different between groups at baseline; however, both parameters were lowered by pioglitazone (23.6%; $p = 0.006$ and 31.7%; $p = 0.008$, respectively), but not by placebo use. Values for the hsCRP decreased 70.9% vs. 0.3% in the pioglitazone and placebo groups, respectively ($p = 0.013$), whereas SAA decreased 34.9% in the pioglitazone and increased 25.5% in the placebo group ($p = 0.029$).

Distribution of HDL subclasses was not different between groups before treatment. Although the proportion of HDL_{2b} was the only subfraction that showed significant increase after pioglitazone treatment (11.9% vs. 13.3%; $p = 0.030$), the percentage change in HDL_{2b}, HDL_{3b}, and HDL_{3c} was different in the pioglitazone versus the placebo group (Figure 2). Pioglitazone favored an increase in HDL particle size (8.80 nm vs. 8.95 nm; $p = 0.044$) and the mean percentage change was significantly different than that observed in the placebo group (+0.77% vs. −0.67%; $p = 0.018$). When HDL composition was analyzed, we observed that only free cholesterol increased significantly after pioglitazone treatment compared with the placebo group (+6.82% vs. −4.87%; $p = 0.043$). HDL-cholesterol-ester showed a tendency to increase in the pioglitazone treatment compared with the placebo group (+5.03% vs. −1.68%; $p = 0.09$). The content of phospholipid and triglycerides, as well as the apolipoprotein content of HDL, was not different at the beginning and at the end of the study.

Spearman correlation analyses between anthropometric measurements, systolic and diastolic blood pressure, activity and duration of SLE, lipid variables, insulin, HOMA-IR, CRP, SAA as well as composition and size of HDL, showed that increases of free cholesterol in HDL particles ($r = 0.573$; $p = 0.002$), HDL-cholesterol plasma concentration ($r = 0.522$; $p = 0.003$), cholesterol-esters in HDL particles ($r = 0.443$; $p = 0.023$), and apo AI ($r = 0.411$; $p = 0.027$), correlated with the increase in HDL particle size.

Discussion

To the best of our knowledge, this prospective randomized controlled trial for the first time indicates significant and potentially important changes in insulin levels, inflammation, HDL-cholesterol and HDL composition as well as subclass distribution in uncomplicated non-diabetic SLE patients during treatment with pioglitazone (3 months; 30 mg/day). These clinical effects, in addition to extensive

Table 2 Lipidic, glucometabolic and inflammation parameters before and after treatment with placebo or pioglitazone

	Placebo (n = *15*)			*Pioglitazone* (n = *15*)		
	Baseline	*Week 12*	p^a	*Baseline*	*Week 12*	p^a
Total Cholesterol (mg/dl)	143 (124–155)	143 (126–159)	NS	133 (119–148)	139 (125–154)	NS
LDL cholesterol (mg/dl)	76 (68–101)	78 (63–103)	NS	79 (52–85)	75 (64–82)	NS
HDL cholesterol (mg/dl)	45 (43–49)	43 (40–57)	NS	46 (37–55)	52 (43–61)	0.029
Triglycerides (mg/dl)	92 (69–182)	91 (66–120)	NS	93 (83–114)	80 (74–120)	NS
Apolipoprotein A (mg/dl)	135 (118–139)	129 (119–149)	NS	130 (121–154)	136 (122–150)	NS
Apolipoprotein B (mg/dl)	62 (55–79)	66 (57–80)	NS	64 (57–78)	62 (57–73)	NS
Fasting glucose (mg/dl)	84 (78–92)	84 (75–90)	NS	85 (78–88)	79 (72–84)	NS
Fasting insulin (μU/mL)	4.92 (0.84–12.95)	3.51 (1.54–12.78)	NS	6.60 (5.11–14.37)	5.40 (3.42–10.34)	0.006
HOMA-IR	1.11 (0.19–2.49)	0.79 (0.32–2.51)	NS	1.50 (1.06–2.80)	1.00 (0.67–1.82)	0.008
hsCRP (mg/l)	1.9 (1.1–3.1)	1.9 (0.9–5.4)	NS	2.6 (0.6–4.2)	0.5 (0.4–2.1)	0.026
Serum amyloid A (mg/l)	3.83 (2.26–9.86)	4.48 (2.75–15.60)	NS	4.97 (1.78–8.39)	2.95 (2.05–7.36)	0.036

Median (interquartile range).
[a] = Wilcoxon test.
LDL, Low Density Lipoprotein; HDL, High Density Lipoprotein; HOMA-IR, homeostasis model assessment-insulin resistance; hsCRP, high sensitivity C reactive protein.

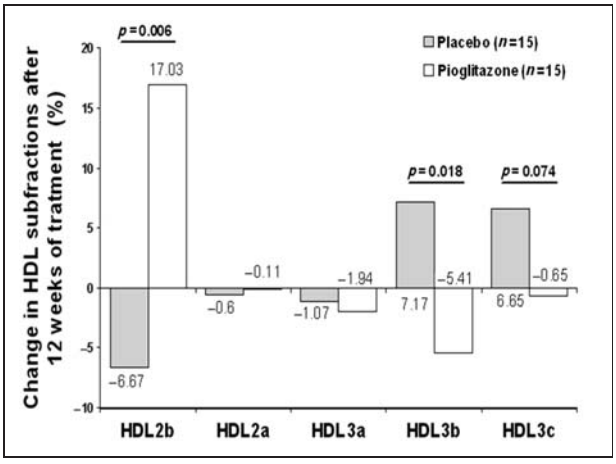

Figure 2 Change in high-density lipoprotein subfraction distributions in patients with systemic lupus erythematosus, after 12 weeks of pioglitazone (30 mg/day) or placebo treatment.

in vitro and in vivo studies assessing anti-inflammatory and anti-atherosclerotic effects of PPAR agonist, indicate that these drugs may be powerful agents in cardiovascular disease prevention, beyond their classical use as regulator for glycemic control.

For a long time, it has been known that patients with lupus present substantially increased morbidity and mortality rates associated with cardiovascular disease. Traditional risk factors, or lupus itself and treatment-related factors, only partly account for the increased risk of CHD in patients with SLE.[1,5] Insulin resistance, defined as the reduced ability of insulin to stimulate glucose uptake in skeletal muscle and fat cells, is an emerging metabolic risk factor that may play a pivotal role in atherogenesis in lupus.[35] We[3] and others[4] have previously shown that patients with SLE presented higher fasting plasma insulin levels as well as reduced insulin sensitivity related to cardiovascular risk factors in SLE patients.[35] The present study shows that pioglitazone administration leads to a significant decrease in insulin levels (23.6%) and HOMA-IR (31.7%). This finding correlates well with a study where a lupus-prone murine model showed improvement of insulin resistance and produced stable glucose levels after pioglitazone administration.[14] Therefore, improving insulin sensitivity in patients at risk due to SLE may prevent the occurrence of cardiovascular complications,

independently of glucose levels, as other investigators have shown in patients with and without diabetes mellitus.[36,37]

Observations that autoimmune and chronic inflammatory disorders are associated with the development of accelerated atherosclerosis have been increasingly recognized.[38] In our study, treatment with pioglitazone significantly decreased hsCRP and SAA by 70.9% and 34.9%, respectively. Previous studies have shown that pioglitazone decreases hsCRP plasma levels.[21] However, to our knowledge, this is the first study where SAA plasma levels were decreased by pioglitazone use. SAA is the major acute-phase protein in vertebrates and is synthesized in the liver in response to infection, inflammation, injury or stress.[39] Although other non-specific inflammatory markers such as hsCRP also correlate with cardiovascular disease, the wider dynamic range as well as the more rapid response and easier measurement of SAA has lead to the suggestion that it may be a better marker of disease,[40,41] and has been shown to predict cardiovascular events.[42,43] Thiazolidinediones such as rosiglitazone or troglitazone reduce SAA by about 30–50% in diabetic and non-diabetic subjects;[44,45] however, only one previous report[46] has evaluated the effect of pioglitazone treatment on SAA. That study[46] showed that patients with diabetes mellitus did not present changes in SAA plasma levels in spite of an improvement in insulin sensitivity. Since inflammation is associated with diabetes mellitus, it is possible that the anti-inflammatory effect of pioglitazone could be more important in patients with SLE, where inflammation plays a central role in the development of atherosclerosis.[47,48] Our results show that in addition to the improvement in insulin resistance, pioglitazone also reduces inflammation in a short time. It is important to highlight that pioglitazone might reduce the risk of CHD in patients with uncomplicated SLE, by reducing the alterations promoted by lupus itself. Longitudinal and epidemiological studies are needed to investigate the effect of this drug over long-term endpoints.

Hypertriglyceridemia and low HDL-cholesterol levels are components of insulin resistance states and of SLE dyslipidemia.[5] In SLE, these lipidic abnormalities correlate with drug therapy, disease activity, metabolic syndrome, and inflammatory cytokines.[1,5,37,49] Consistent with a previous study,[9] we found that HDL-cholesterol and triglyceride levels were normal in our group of patients, possibly due to the fact that we selected subjects with uncomplicated SLE. Treatment with pioglitazone raised HDL-cholesterol levels by 14.2%, similar to previous studies where pioglitazone increased HDL-cholesterol levels by ~10–20%.[21] It has been suggested that PPAR signaling may play a role in stimulating expression of the gene encoding ABCA1,[50] which could increase the flux of cholesterol from cells on to apo AI.[51] If PPAR were involved in regulating ABCA1 gene expression, additional effects on plasma HDL-cholesterol levels and HDL particles might be seen.

Although it is a widely accepted notion that low HDL-cholesterol levels constitute an independent risk factor for premature atherosclerosis and CHD, there is increasing evidence that HDL particle characteristics may be more important than quantity for atheroprotection.[15] We[6] and others[7] have previously demonstrated that compared with control subjects, patients with SLE have triglyceride enrichment and cholesterol-ester depletion of HDL, as well as low concentrations of large HDLs and high levels of small HDL particles. These abnormal HDL particles have been found in other hyperinsulinema states, and have been associated with coronary heart disease[52] and recurrence of coronary events.[12] In fact, McMahon et al. reported that 48.2% from 276 SLE patients have proinflammatory HDLs, and that these proinflammatory HDLs contribute to a 17-fold increased odds for the presence of atherosclerosis.[9] Our results show that pioglitazone modified HDL particle size and composition, and although we did not evaluate HDL atheroprotective functionality after pioglitazone use, several studies have shown that small HDL particles and HDLs enriched in triglycerides or depleted of cholesterol show a diminished atheroprotective function and are associated with a poor prognosis for cardiovascular disease.[12,15,52] Therefore, our findings suggest that pioglitazone could restore the HDL atheroprotective functions in patients with SLE.

It is important to note that the small sample size may explain the lack of significant differences in plasma apo AI levels and HDL composition after pioglitazone treatment. However, the 15 patients included in the pioglitazone arm were enough to detect changes in insulin, inflammation and HDL particle size. The clinical implications of these findings are that even in SLE patients without additional risk factors, representing approximately 15% of our cohort, pioglitazone may have the potential to prevent premature CHD. We do not have hard endpoints in our study to confirm if all these qualitative and quantitative changes are reflected in a prognostic improvement of patients with SLE. Longitudinal studies are necessary to

evaluate the cardiovascular effects of long-term pioglitazone use in SLE patients with and without diabetes mellitus and other complications.

In conclusion, the findings of our study indicate that pioglitazone treatment may exert multiple beneficial cardiovascular effects in uncomplicated normoglycemic SLE patients. This drug significantly enhanced insulin sensitivity, reduced inflammation and modified HDL characteristics, suggesting that PPAR activation may lead to potential beneficial effects in patients with uncomplicated SLE at risk of cardiovascular disease.

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Conflict of interest

The authors declare that they have no conflicts of interest.

Other information

The trial is registered at ClinicalTrials.gov, number NCT01322308.

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