Effects of High-dose Atorvastatin on Antiinflammatory Properties of High Density Lipoprotein in Patients with Rheumatoid Arthritis: A Pilot Study

CHRISTINA CHARLES-SCHOEMAN, DINESH KHANNA, DANIEL E. FURST, MAUREEN McMAHON, SRINIVASA T. REDDY, ALAN M. FOGELMAN, HAROLD E. PAULUS, GRACE S. PARK, TIMOTHY GONG, and BENJAMIN J. ANSELL

ABSTRACT. ***Objective.*** Patients with rheumatoid arthritis (RA) have a 2–3-fold increased risk of myocardial infarction. Recent work suggests that plasma high density lipoproteins (HDL) from patients with RA are more proinflammatory than HDL from controls. We examined the effects of atorvastatin 80 mg daily on the inflammatory properties of HDL and clinical disease activity in RA.

Methods. Twenty subjects with active RA (mean Disease Activity Score 5.13 ± 0.92) without dyslipidemia and no history of coronary artery disease were randomized in a double-blind placebo-controlled trial to receive 80 mg of atorvastatin (A) or placebo (P) daily in addition to stable antirheumatic drug therapy. Disease activity variables were followed over 12 weeks and the anti-/proinflammatory properties of HDL were determined by a cell-free assay (CFA) that measures lipid oxidation products.

Results. After 12 weeks, subjects completing the A protocol had a mean reduction in CFA values of 14.8 ± 21.7%, while subjects completing P protocol had a mean increase in CFA values of 7.1 ± 13.2% ($p = 0.026$). There was a trend for a decrease in highly sensitive C-reactive protein (hs-CRP) over 12 weeks in the A group compared to an increase in hs-CRP in the P group ($p > 0.05$), but changes in measures of clinical disease activity and plasma cytokine/intercellular adhesion molecule-1 levels were not significantly different in the A and P groups.

Conclusion. In patients with active RA, HDL was rendered more antiinflammatory by high-dose atorvastatin compared to placebo. Functional characterization of HDL may warrant further investigation as a method of cardiovascular risk assessment in RA patients without traditional coronary risk factors. (ClinicalTrials.gov number NCT00356473). (First Release June 1 2007; J Rheumatol 2007;34:1459–64)

Key Indexing Terms:
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Premature cardiovascular disease has been recognized as a major comorbidity in patients with rheumatoid arthritis (RA), who on average die 3–18 years earlier than members of the general population[1]. It is reported to be the most common cause of death, occurring in 42% of patients with RA[2]. In a prospective cohort study involving 114,342 women, those with RA had a > 2-fold higher risk of myocardial infarction (MI) than women without RA. Women with RA for at least 10 years had a > 3-fold higher risk of MI. This increased risk of MI persisted even after controlling for known coronary risk factors including hypertension, hyperlipidemia, diabetes mellitus, tobacco use, and body mass index[3]. Greater insight into the relationship between RA and atherosclerosis may allow for development of therapeutic strategies to minimize the clinical consequences of both conditions.

The initiation of the atherosclerotic plaque occurs when low density lipoprotein (LDL) cholesterol particles are transported into the artery wall and oxidized. Oxidation of LDL leads to inflammation in the artery wall and progression of atherosclerotic plaque by attracting and enhancing migration of inflammatory cells into the artery wall[4-6].

Under normal conditions, high density lipoproteins (HDL) inhibit LDL oxidation, lessen monocyte migration, and promote cholesterol efflux from artery wall cells, thus retarding the growth of atheromata[4,7-10]. Further, HDL-cholesterol (HDL-C) levels are inversely related to risk for atherosclerotic events[11].

During an acute-phase response and in the presence of chronic inflammation, HDL's atheroprotective capacity can be

From the Division of Rheumatology, University of California, Los Angeles David Geffen School of Medicine, Los Angeles, California, USA.

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C. Charles-Schoeman, MD; D.E. Furst, MD; M. McMahon, MD, MS; H.E. Paulus, MD; G.S. Park, MPH, Division of Rheumatology; S.T. Reddy, PhD; A.M. Fogelman, MD; T. Gong, BA; B.J. Ansell, MD, Division of Cardiology, Atherosclerosis Research Unit, UCLA David Geffen School of Medicine; D. Khanna, MD, MS, Division of Immunology, University of Cincinnati, Cincinnati, Ohio, USA.

Address reprint requests to Dr. C. Charles-Schoeman, David Geffen School of Medicine at UCLA, 1000 Veteran Avenue, 32-59 Rehab, Box 951670, Los Angeles, CA 90095-1670. E-mail: ccharles@mednet.ucla.edu

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compromised[12-14]. Van Lenten, *et al* first demonstrated that HDL had the potential to be antiinflammatory or proinflammatory depending on the inflammatory milieu[14]. During the acute-phase response in rabbits and humans, HDL loses its ability to prevent LDL-induced monocyte chemotaxis and paradoxically promotes LDL-induced inflammation. The authors concluded that under basal conditions, HDL functions in an antiinflammatory capacity; however, during the acute-phase response there is displacement and/or exchange of apoliprotein A-I and other proteins associated with HDL, resulting in loss of antioxidant enzyme activity, and proinflammatory HDL[14]. The anti- or proinflammatory activity of HDL was measured by a cell-based monocyte chemotaxis assay, (MCA), in which HDL is added to standard human LDL in an artery wall coculture system, as described[14]. HDL that increases monocyte chemotaxis relative to LDL alone is defined as proinflammatory, whereas HDL that decreases monocyte chemotaxis is considered antiinflammatory[14].

Ansell and colleagues reported that a cohort of non-RA patients with known coronary artery disease (CAD) and/or coronary risk equivalents had significant HDL dysfunction or "proinflammatory HDL" relative to healthy controls. This HDL paradoxically enhanced LDL oxidation and promoted chemotaxis of monocytes[15]. Another group of patients with CAD who had particularly high serum levels of HDL-C (> 84 mg/dl) also exhibited proinflammatory HDL in the setting of documented CAD[15]. Both the monocyte chemotaxis assay and a cell-free assay (CFA) were used to evaluate HDL antiinflammatory activity in this study, with excellent correlation between the 2 assays (correlation coefficient = 0.751; p = 0.00009)[15].

McMahon and colleagues studied the pro/antiinflammatory properties of HDL from patients with systemic lupus erythematosus and RA. Patients with these 2 diseases had a significantly higher incidence of proinflammatory HDL when compared to healthy controls as measured by the CFA[16].

It is appealing to consider therapeutic approaches that may act to reverse this HDL dysfunction in patients with RA. Hydroxymethylglutaryl-coenzyme A reductase inhibitors (statins) are widely used lipid-lowering agents that have also been reported to display multiple antiinflammatory and immunomodulatory activities *in vitro*[17,18]. Although results of statin treatment studies in animal models of inflammatory arthritis have been mixed[17,19,20], a placebo-controlled trial of atorvastatin 40 mg daily in 116 patients with RA demonstrated mild but clinically apparent antiinflammatory effects on joint inflammation[21].

We reported a modest, but statistically significant improvement in HDL antiinflammatory properties with simvastatin 40 mg daily in patients with coronary heart disease (CHD) or CHD risk equivalent[15]. We undertook this pilot study to examine the effects of a statin on HDL antiinflammatory properties in the setting of chronic, active inflammation due to RA. The effects of high-dose atorvastatin at 80 mg daily on HDL antiinflammatory properties and clinical disease activity were evaluated in patients with RA but without coronary disease.

MATERIALS AND METHODS

Patients. Patients were recruited from the rheumatology offices at University of California, Los Angeles (UCLA) via flyers posted in the offices and in the UCLA Medical Center. All patients gave written informed consent for the study under a protocol approved by the Human Research Subject Protection Committee at UCLA.

Participants met the American College of Rheumatology (ACR) criteria for RA, were at least 18 years of age, had RA for at least 1 year with ongoing active disease, and were taking stable doses of disease modifying antirheumatic drug (DMARD) therapy for at least 3 months prior to study entry. Active disease was defined as at least 2 of: (1) ≥ 6 tender joints; (2) ≥ 3 swollen joints; (3) ≥ 45 min of morning stiffness. Exclusion criteria included inability to give informed consent, pregnancy or lactation, eligibility for pharmacologic lipid-lowering therapy per National Cholesterol Treatment Program Adult Treatment Panel III guidelines, use of any lipid-lowering medication, known hepatic disease or elevated liver transaminase levels within the past 2 months, and previous treatment in the last 3 months with hydroxychloroquine.

Study protocol. Patients continued taking stable doses of pre-study DMARD, nonsteroidal antiinflammatory drugs (NSAID), and prednisone during the study. Patients were randomly allocated to either atorvastatin 80 mg or placebo for a total of 12 weeks. Computerized randomization was done by the UCLA research pharmacy, and neither patients nor doctors were aware of drug allocation. Atorvastatin (2 pills of 40 mg) and matching placebo tablets were kindly provided by Pfizer.

After screening, patient followup visits occurred at 0, 3, 6, 12, and 18 weeks. Fasting blood was collected at 0, 6, and 12 weeks for lipid analysis and liver function testing; HDL antiinflammatory activity using the CFA was assessed at Weeks 0 and 12. Highly sensitive C-reactive protein (hs-CRP) was measured at 0, 3, 6, 12, and 18 weeks and Westergren erythrocyte sedimentation rate (ESR) and interleukin 6 (IL-6), tumor necrosis factor-α (TNF-α), and intercellular adhesion molecule-1 (ICAM-1) plasma levels were measured at 0 and 12 weeks. MultiAnalyte Profile testing (Rules-Based Medicine, Austin, TX, USA) was used for cytokine and adhesion molecule analysis.

Prespecified primary outcomes were change in HDL antiinflammatory properties and hs-CRP after 12 weeks of therapy. Secondary outcomes included change in Disease Activity Score using a 28 joint count (DAS28), patient and physician global assessments on visual analog scale (VAS; 0–100), swollen and tender joint counts, patient pain assessment on VAS (0–100), ESR, cytokine/ICAM-1 levels, lipid levels, and the Health Assessment Questionnaire Disability Index (HAQ-DI).

The same researcher measured all clinical variables at study entry and at followup visits and was blinded to all laboratory testing. Adverse events were recorded throughout the study and solicited by specific request at a 1-week telephone call and at all followup assessments. (ClinicalTrials.gov number NCT00356473).

HDL antiinflammatory analysis by CFA. The CFA was a modification of a published method[22] using LDL as the fluorescence-inducing agent. Control LDL was prepared as described[22]. HDL-containing supernatants were first isolated by the dextran sulfate method. 50 μl of HDL Magnetic Bead Reagent (Polymedco catalog no. 5030) were mixed with 250 μl of patient plasma and incubated for 5 min at room temperature. The solution was then incubated for an additional 5 min on a magnetic particle concentrator. HDL-C in the supernatant was quantified using a standard assay (Thermo DMA Co., San Jose, CA, USA). To determine the antiinflammatory properties of HDL, the change in fluorescence intensity as a result of the oxidation of dihydrodichlorofluorescein (DCFH) by a standard normal control LDL in the absence or presence of the test HDL was assessed. DCFH diacetate (DCFH-DA) was first dissolved in fresh methanol at 2.0 mg/ml and incubated in the dark at room temperature for 20 min, resulting in release of DCFH. 25 μl of LDL-C (100 μg/ml) was mixed with 6.25 μl of test HDL (100 μg HDL-C/ml) in black, flat

bottom polystyrene microtiter plates and incubated at 37°C with rotation for 30 min. 25 μl of DCFH solution (0.2 mg/ml) was then added to each well, mixed, and incubated at 37°C for 1 h with rotation. Fluorescence was determined with a plate reader (Spectra Max, Gemini XS; Molecular Devices, Sunnyvale, CA, USA) at an excitation wavelength of 485 nm, emission wavelength of 530 nm, and cutoff of 515 nm with photomultiplier sensitivity set at medium. Values for intra- and interassay variability were 0.5 ± 0.37% and 3.0 ± 1.7%, respectively.

Statistical analysis. Data were analyzed using SAS Release 8.02 (SAS Institute Inc., Cary, NC, USA). Patient groups were compared using Student's t-test for continuous variables and the chi-square test for association for categorical variables, along with Fisher's exact test for small sample sizes. When needed, nonparametric Wilcoxon rank-sum tests were used for continuous variables. The significance level was prespecified at $p < 0.05$.

RESULTS

Patient demographic and clinical characteristics. Thirty subjects were screened and 20 participants who met study criteria were randomly assigned to either atorvastatin or placebo. The most common reasons for exclusion were change in DMARD therapy within 3 months prior to screening and lack of sufficient disease activity. Subjects were also excluded if they had a history of coronary disease or coronary risk equivalents, or were otherwise candidates for lipid-lowering therapy as defined by the National Cholesterol Education Program. Randomization produced demographic and clinical characteristics that were similar in the 2 groups with the exception of hs-CRP levels, which were significantly higher in the atorvastatin group compared to the placebo group ($p = 0.04$; Table 1). All participants had active, chronic RA with a mean duration of 16 years. Baseline HDL antiinflammatory properties determined by the CFA, traditional cardiac risk factors, and regular aspirin use were similar between the 2 groups (Table 1). All participants were taking DMARD therapy and use of prednisone, nonselective NSAID, and cyclooxygenase-2 selective inhibitors were comparable (Table 2).

After 12 weeks of therapy, there were significant reductions in both total cholesterol and LDL-C in the atorvastatin group compared to placebo ($p < 0.0001$; Table 3). Although there were no significant changes in HDL-C concentrations in

Table 1. Baseline characteristics.

Characteristics	Placebo (n = 9)	Atorvastatin (n = 11)	p
Demographic			
Age (yrs)	53 ± 10	58 ± 12	0.31
Sex (F)	89% (8/9)	100% (11/11)	0.45
Rheumatoid factor-positive	90% (10/11)	67% (6/9)	0.28
Disease duration (yrs)	15.5 ± 14.3	16.8 ± 9.8	0.86
Clinical outcome measures and inflammatory markers			
hs-CRP (mg/l)	3.2 ± 3.5	10.4 ± 9.4	0.04
ESR (mm/h)	15.7 ± 12.5	19.6 ± 10.9	0.46
68 Tender joints	26.7 ± 14.1	25.0 ± 10.5	0.85
66 Swollen joints	8.6 ± 2.4	10.7 ± 3.7	0.15
DAS28	5.1 ± 1.0	5.3 ± 1.1	0.47
HAQ (disability)	1.29 ± 0.69	1.40 ± 0.87	0.77
Patient global	47 ± 24	56 ± 27	0.41
Physician global	50 ± 19	45 ± 11	0.57
Patient pain	44.2 ± 22.9	54.2 ± 28.2	0.40
IL-6 (pg/ml)	9.8 ± 7.0	23.3 ± 31.2	0.47
TNF-α (pg/ml)	7.5 ± 7.1	7.6 ± 7.1	0.97
ICAM-1 (ng/ml)	183.1 ± 47.0	208.8 ± 64.2	0.33
HDL antiinflammatory properties (CFA) and lipids			
CFA (fluorescence units)	716.4 ± 276.9	784.4 ± 238.6	0.56
Total-C (mg/dl)	201 ± 27	191 ± 33	0.46
HDL-C (mg/dl)	63 ± 10	64 ± 17	0.86
LDL-C (mg/dl)	118 ± 31	110 ± 28	0.50
TG (mg/dl)	100 ± 36	92 ± 47	0.67
Traditional cardiovascular risk factors, %			
Family history	22 (2/9)	36 (4/11)	1.00
Hypertension	33 (3/9)	36 (4/11)	1.00
Smoking	0	18 (2/11)	0.49
Diabetes	0	0	1.00
Aspirin use	11 (1/9)	27 (3/11)	0.60

Data are mean ± standard deviation. For categorical variables, chi-square tests including Fisher's exact tests for small sample sizes were used. For continuous variables, t-tests were used for parametric data, and Wilcoxon rank-sum tests for nonparametric data. hs-CRP: highly sensitive C-reactive protein; ESR: erythrocyte sedimentation rate (Westergren); DAS28: disease activity scale using a 28 joint count; HAQ: Health Assessment Questionnaire; ICAM-1: intercellular adhesion molecule-1; TNF-α, tumor necrosis factor-α; CFA: cell-free assay; Total-C: total cholesterol; HDL: high density lipoprotein; LDL: low density lipoprotein; TG: triglycerides.

Table 2. Concomitant DMARD, prednisone, and NSAID/COX-2 inhibitor therapy.

	Placebo (n = 9), %	Atorvastatin (n = 11), %	p
DMARD	100 (9/9)	100 (11/11)	1.00
Methotrexate	77 (7/9)	55 (6/11)	0.37
TNF inhibitor	55 (5/9)	64 (7/11)	1.00
Leflunomide	11 (1/9)	0 (0/11)	0.45
Sulfasalazine	0 (0/9)	18 (2/11)	0.48
Nonselective NSAID	55 (5/9)	27 (3/11)	0.36
COX-2 inhibitor	44 (4/9)	36 (4/11)	1.00
Prednisone	0 (0/9)	27 (3/11)	0.22

DMARD: disease modifying antirheumatic drugs; NSAID: nonsteroidal antiinflammatory drugs; COX-2: cyclooxygenase-2.

Table 3. Mean differences in primary and secondary outcomes after 12 weeks of therapy.

Outcomes	Placebo (n = 9)	Atorvastatin (n = 11)	p
Primary outcomes			
CFA (fluorescence units)	+ 49 (–159 to 204)	–135 (–490 to 199)	0.026
hs-CRP (mg/l)	+ 4.8 (–3.5 to 41.2)	–5.6 (–24.9 to 1.3)	0.14
Secondary outcomes			
Total-C (mg/dl)	+ 1.0 (–32 to 57)	–63.1 (–106 to –40)	< 0.0001
HDL-C (mg/dl)	–0.8 (–0.9 to 7.0)	–2.0 (–14.0 to 16.0)	0.76
LDL-C (mg/dl)	+ 0.8 (–23 to 45)	–57.9 (–93 to –37)	< 0.0001
TG (mg/dl)	+ 4.5 (–53 to 101)	–16.8 (–86 to 22)	0.33
ESR (mm/h)	0 (–10 to 19)	–2.7 (–28 to 14)	0.64
68 Tender joints	–10.1 (–34 to 32)	–12.0 (–37 to 1)	0.81
66 Swollen joints	–3.8 (–7 to 5)	–0.4 (–9 to 20)	0.11
DAS28	–0.78 (–2.5 to 2.2)	–0.80 (–3.9 to 1.1)	0.98
HAQ (disability)	–0.13 (–0.50 to 0.63)	–0.33 (–1.38 to 0.38)	0.40
Patient global	+ 3.0 (–33 to 32)	+ 0.4 (–45 to 61)	0.85
Physican global	–15.3 (–37 to 22)	–11.6 (–64 to 16)	0.74
Patient pain	+ 3.4 (–37 to 33)	–0.3 (–63 to 63)	0.80
IL-6 (pg/ml)	–0.2 (–11.2 to 12.6)	–10.7 (–71.6 to 1.9)	0.46
TNF-α (pg/ml)	+ 9.0 (–2.3 to 61.9)	+ 1.1 (–2.5 to 5.7)	0.96
ICAM-1 (ng/ml)	+ 10 (–37 to 57)	+ 15 (–46 to 127)	0.80

Range of values given in parentheses. CFA: cell-free assay; TG: triglycerides. For other abbreviations, see Table 1.

either group, there was a significant 14.8% improvement in HDL antiinflammatory activity in the atorvastatin group compared to a 7.1% worsening in the placebo group (p = 0.026).

There was a trend for a decrease in hs-CRP levels over 12 weeks in the atorvastatin group, compared to an increase in hs-CRP levels in the placebo group. While there was an initial statistically significant improvement at 3 weeks in hs-CRP in the atorvastatin group compared to placebo group (p = 0.026; data not shown), the statistical significance was not maintained throughout the 12 weeks of the study. In general, slight to modest improvements were noted in both the placebo and the atorvastatin groups, but the changes in clinical outcomes measures were not clinically or statistically significant and there were no differences between the groups after 12 weeks of therapy (Table 3). Similarly, changes in TNF-α, IL-6, and ICAM-1 after 12 weeks of treatment were not significantly different, comparing the placebo and atorvastatin groups (Table 3).

Both atorvastatin 80 mg daily and placebo were well tolerated in the study cohort, and no serious adverse events occurred during the study period. One member of the placebo group complained of myalgias, but had a normal creatinine kinase level, with resolution of myalgias after the study drug was held for a few days. Treatment resumed without further complaints. No liver transaminase elevations above 2.5 times the upper limit of normal (ULN) occurred in any participants, but 2 members of the atorvastatin group had mild elevations below 2.5 times ULN. One participant in each group declined followup testing, the first in the placebo group citing time and transportation problems, the second in the atorvastatin group out of concern regarding minor elevation (< 2.5× ULN) in transaminase levels, which resolved completely after the study drug was held for 2 days. A second patient with mild transaminase elevation at an early study followup visit had normalization of transaminases without interruption of study drug administration.

DISCUSSION

Despite the success of new treatments in improving the quality of life for more patients with RA, their effects on atherogenesis are largely unknown. Although several studies[23-25] have suggested that antirheumatic treatment may be associated with reduced mortality, only one study has taken into account severity of disease in the analysis[26]. Further, recent work suggests that longterm infusion of infliximab, an anti-TNF-α agent, is associated with a proatherogenic lipid profile, in particular a decrease in HDL-C[27].

Abnormal antiinflammatory HDL properties, or "proinflammatory" HDL, have been linked to the presence of CHD in members of the general population[15,22]. The antiinflammatory properties of HDL appear to distinguish patients from control subjects better than HDL-C levels themselves[15]. Recent evidence suggests that proinflammatory HDL is more common in patients with RA than in healthy controls[16].

Longterm and short-term trials with statins have shown significant reductions in cardiovascular events in patients with and without history of CHD[28-34]. A large primary prevention study of 3500 patients with RA is currently under way to determine if statins will also decrease morbidity and mortality from CHD in patients with RA. A previous report of clinically apparent antiinflammatory effects of atorvastatin in patients with RA (TARA trial) makes use of statins particularly attractive in RA[21].

While previous work has shown that simvastatin can improve HDL antiinflammatory properties in patients with known CHD[15], our current study is the first to demonstrate the ability of statins to improve HDL antiinflammatory properties in patients with a chronic, autoimmune, inflammatory disease such as RA. While the study's interpretation must be limited by its small sample size, our investigation does suggest a novel rationale for cardiovascular risk reduction with use of statins in this population.

Our patients had modestly elevated hs-CRP levels that tended to decrease in the atorvastatin group. While elevated hs-CRP is an accepted risk factor for CHD in the general population[35,36], interpretation of elevated levels in patients with systemic inflammation from RA may be confounded by disease activity. Some studies suggest that higher systemic levels of inflammation as measured by ESR may correlate with increased CHD mortality[37].

Changes in measures of clinical disease activity and plasma cytokine/ICAM-1 levels did not differ significantly between the atorvastatin and placebo groups. Given the pilot design of the study with susceptibility to type II error and previous results with 40 mg of atorvastatin in the TARA trial, we hypothesize that this reflects our small study population. It should also be noted that patients were followed for 3 months in contrast to the 6 month duration of the TARA trial. Even at its highest approved dosage of 80 mg, atorvastatin was well tolerated in our population, all of whom were taking some form of DMARD therapy, including 65% who were taking methotrexate.

Atorvastatin modestly but significantly improved HDL antiinflammatory properties in patients with chronic, active RA. Although these results must be confirmed with larger-scale studies, proinflammatory HDL appears to be an emerging coronary risk factor that is more common in patients with RA and may be safely improved with statin therapy.

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