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Associations between disease activity, markers of HDL functionality and arterial stiffness in patients with rheumatoid arthritis

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ABSTRACT

Background and aims: Rheumatoid arthritis (RA) is a chronic, inflammatory disease associated with increased risk of cardiovascular disease (CVD). Measures of HDL metabolism/function were shown to be altered in RA patients with high disease activity. We aimed at evaluating the effect of HDL characteristics on arterial stiffness in RA patients classified according to the inflammatory disease activity.
Methods: RA patients were classified according to disease activity (DAS-28) into active RA (n = 27; DAS-28 > 3.2) and inactive RA patients (n = 17; DAS-28 < 3.2). A control group of healthy individuals was also studied (n = 33). Clinical and biochemical characteristics, cholesteryl ester transfer protein (CETP) and paraoxonase 1 (phenylacetate and paraoxonase) activities and carotid-femoral pulse wave velocity (cf-PWV) were determined.
Results: Anthropometric characteristics were similar in all groups. In accordance with the inflammatory status, active RA patients presented elevated hsCRP levels ($p < 0.001$). There were no differences in the lipid profile between groups. Similarly, features of insulin resistance were absent in RA patients (p = non-significant). Active RA patients presented higher CETP activity than the other two groups ($p = 0.026$). Phenylacetate and paraoxonase activities were altered in active RA patients in comparison with the other groups ($p = 0.034$ and $p = 0.041$, respectively). Cf-PWV was significantly higher in active RA patients in comparison with controls, following adjustment by age ($p = 0.030$). Age ($\beta_{st} = 0.468$, $p = 0.013$) and apo A-I levels ($\beta_{st} = -0.405$, $p = 0.029$) were independent predictors of cf-PWV in a model including hsCRP, HOMA-IR, and phenylacetate activity ($r^2 = 0.42$).
Conclusions: High DAS-28 identifies patients with alterations in HDL characteristics. Plasma levels of apo A-I can be used as a marker of arterial stiffness in RA.

1. Introduction

Rheumatoid arthritis (RA) is a chronic, systemic inflammatory disease associated with increased mortality and morbidity, predominantly as a result of cardiovascular disease (CVD) [1]. An approximately 2-fold increase in mortality from myocardial infarction and stroke has been observed in studies comparing RA patients with the general population [1–3]. Such increased risk of CVD cannot be completely explained by traditional risk factors [4], and, therefore, cannot be fully predicted by the conventional CVD risk markers.

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Dyslipidemia is not frequent in RA patients [5,6] and, if present, the most common lipid alteration involves low levels of high-density lipoprotein-cholesterol (HDL-C) concentration. Nonetheless, despite the presence or absence of low HDL-C, HDL functionality can be altered in RA patients. Recently, it was shown that HDL cholesterol efflux capacity was associated with subclinical atherosclerosis [7] and incident cardiovascular events independently of HDL-C levels [8]. Therefore, metrics of HDL functionality can be more informative than lipoprotein levels. The main limitation is that HDL functionality assays are laborious and hard to standardize. Up to the moment, none of the available assays are suitable for their use in the general practice. In its place, surrogate metrics of HDL functionality, such as the concentration of apolipoprotein (apo) A-I [9], the major functional and structural component of HDL, and the activity of paraoxonase 1 (PON 1) can be relevant. Such analysis and its impact on measurements of vascular compliance and inflammation have been scarcely studied in RA.

PON 1 is an antioxidant enzyme exclusively associated with HDL which plays a major function in preventing lipid oxidation [10]. PON 1 is important for the protection of endothelial cells from damage derived from oxidized phospholipids [10]. RA patients presented lower PON 1 activity than controls even in the absence of changes in the lipid profile [11,12]. Such decrease was associated with inflammation and was partially reversed by treatment with biological agents [13].

Cholesteryl ester transfer protein (CETP) is a protein which mediates the transfer of neutral lipids (cholesteryl esters and triglycerides) between HDL and apo B-containing lipoproteins. Therefore, CETP is one of the determinants of HDL chemical composition and HDL plasma subfraction distribution, which, in turn, influence HDL functionality [14]. Therefore, elevated CETP activity can be a factor associated with HDL dysfunction in RA, as it has been demonstrated in patients with metabolic syndrome and type 2 diabetes [15,16].

Carotid to femoral pulse wave velocity (cf-PWV) is a measure of vascular compliance which correlates with active inflammation and has been associated with increased CVD risk in RA [17,18]. Accordingly, cf-PWV was increased in RA patients *vs.* controls [17,18], and this alteration was modified by treatment with biological agents [19,20]. However, the relationship between metrics of HDL functionality and cf-PWV has not been assessed yet. The aim of the present study was to evaluate the effect of HDL characteristics on arterial stiffness in RA patients classified according to the disease inflammatory activity.

2. Materials and methods

2.1. Subjects

Forty four patients with clinical manifestations of RA were recruited at José de San Martín Clinical Hospital, University of Buenos Aires, and at Buenos Aires Italian Hospital (Buenos Aires, Argentina) from April 2009 to January 2010. All patients met the 1987 revised RA criteria of the American College of Rheumatology [21]. RA patients were classified according to the disease activity score using 28 joint count and the erythrocyte sedimentation rate (DAS-28) [22]. Moderate-high disease activity was defined as a DAS-28 score >3.2 (Active RA, n = 27) and low disease activity as a DAS-28 score <3.2 (Inactive RA, n = 17).

Among the group of active RA patients, 10 patients were on methotrexate (MTX) monotherapy and 14 were on DMARDs combination therapy. Six patients were receiving MTX + hydroxychloroquine (HCQ), 6 were on MTX + tumor necrosis factor (TNF) inhibitor therapy, one was taking MTX + a pyrimidine synthesis inhibitor and one was on MTX + rituximab + HCQ. Finally, one patient was taking a pyrimidine synthesis inhibitor as monotherapy, one TNF inhibitor therapy as monotherapy and one patient was treatment naïve. Among the group of inactive RA patients, 9 of them were on MTX monotherapy and 6 were on DMARD combination. Four patients were on MTX + HCQ and 2 were taking a pyrimidine synthesis inhibitor + HCQ. Of the rest of the patients, one was on TNF inhibitor therapy as a monotherapy and one was treatment-naïve. Importantly, there were no significant differences in the treatment modalities between the groups (Supplementary Table 1). In addition, there were no differences in the consumption of nonsteroidal anti-inflammatory drugs (NSAIDs) between active and inactive RA patients (15/27 *vs.* 6/17; $p = 0.124$). Biological agents were administered to 8/27 and 1/17 of active and inactive RA patients, respectively ($p = 0.121$), mostly as an add-on therapy to MTX. Corticoids were only taken by active RA patients (8/27 *vs.* 0/17, $p = 0.015$). Patients were not advised to suspend medication prior to the study to accurately evaluate the balance between atherogenic and antiatherogenic factors. No medication changes were registered in a two-week period prior to the blood extraction.

Exclusion criteria for the patient selection were: presence of infectious diseases, diabetes or any previous cardiovascular event, treatment with drugs capable of producing vasculitis or cardiac dysfunction and use of antioxidants and/or lipid-lowering drugs during the last month. Thirty three healthy, normolipidemic, middle-aged subjects were recruited to form a control group. Written informed consent was obtained from all participants and the study was approved by the Ethics Committees from School of Pharmacy and Biochemistry, University of Buenos Aires, and from the Buenos Aires Italian Hospital in accordance with local institutional guidelines conformed to the Declaration of Helsinki.

2.2. Blood samples

Blood samples were withdrawn from the antecubital vein of each participant at the time of recruitment after 12 h overnight fast. Serum and EDTA plasma (final EDTA concentration: 1 mg/ml) were prepared from venous blood collected into sterile, evacuated tubes (BD, Vacutainer®). Plasma was immediately separated by low-speed centrifugation at 4 °C; serum and plasma were aliquoted and frozen at −80 °C under nitrogen; each aliquot was thawed only once directly before analyses.

2.3. Clinical and biological parameters

Complete blood count was determined in a Coulter® GEN-S autoanalyser (Beckman Coulter, Fullerton, CA, USA.). Erythrocyte sedimentation rate (ESR) was determined using Test 1 analyser (Alifax, Padova, Italy). Plasma levels of total cholesterol (TC), triglycerides (TG), glucose, urea, uric acid, creatinine, albumin, aspartate amine transferase (AST), alanine amine transferase (ALT) and alkaline phosphatase (ALP), were measured by standardized methods in a COBAS® C501 autoanalyser (Roche, Mannheim, Germany). LDL-cholesterol (LDL-C) and HDL-cholesterol (HDL-C) were determined by selective precipitation methods. Plasma apo A-I, apo B and high-sensitivity C-reactive protein (hsCRP) were quantitated by immunoturbidimetry (Roche, Mannheim, Germany). Serum amyloid A (SAA) and rheumatoid factor (RF) were determined by nephelometry (Siemens, Munich, Germany) and insulin levels by radioimmunoassay (DPC, Los Angeles, California, USA). Antibodies anti-cyclic citrullinated peptides (Anti-CCP) were measured by a 2nd generation immunoassay (INOVA Diagnostics, San Diego, CA, USA).