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Structural and functional changes in HDL with low grade and chronic inflammation

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ABSTRACT

Objective: HDL functionality has been shown to be impaired in inflammatory conditions, including coronary artery disease. The present study aims to determine the impact of low grade and acute inflammation on HDL function and structure.

Approach and results: i) The endothelial protective effects of HDL were compared between 26 periodontal patients and 26 age and sex matched controls by measuring paraoxonase activity in serum and nitric oxide bioavailability and superoxide production in endothelial cells. Paraoxonase activity and nitric oxide bioavailability were reduced, while superoxide production was increased ($p < 0.01$) in periodontal patients compared to controls. ii) HDL function, including cholesterol efflux and vascular cell adhesion molecule-1 expression, was subsequently measured in the periodontal patients following an inflammatory stimulus. There was an acute deterioration in HDL's endothelial protective function, without change in cholesterol efflux, after 24 h ($p < 0.01$ for all). These functional changes tracked increases of inflammatory markers and altered HDL composition. Finally, HDL function returned to baseline levels after resolution of inflammation.

Conclusion: This study demonstrates that even minor alterations in systemic inflammation can impair the endothelial protective effects of HDL. These functional changes were independent of cholesterol efflux and were associated with remodeling of the HDL proteome. All measures of HDL's endothelial protective functions recovered with resolution of inflammation. These findings suggest that HDL dysfunction may represent a novel mechanism linking inflammation with progression of atheroma.

1. Introduction

Epidemiological studies have shown that high-density lipoprotein (HDL) cholesterol is a strong predictor of cardiovascular (CV) risk, with increments of 1 mg/dL in serum HDL levels being associated with a 3–4% reduction in mortality [1,2]. The traditional mechanism by which HDL exerts its 'atheroprotective' effect has been considered to be reverse cholesterol transport from the arterial wall. However, a number of alternative 'anti-atherogenic' properties have been ascribed to HDL, including stimulation of endothelial nitric oxide (NO), inhibition of reactive oxygen species as well as anti-thrombotic mechanisms. It may also promote endothelial progenitor cell mediated vascular repair and have a central role in the regulation of the immune system [3–8].

In both experimental and clinical studies, these beneficial properties can be lost and HDL may acquire a 'pro-inflammatory' and 'pro-oxidant' phenotype, which may be relevant to CV risk. We have reported that HDL, isolated from patients with inflammatory conditions, such as diabetes and anti-phospholipid syndrome, impairs nitric oxide bioavailability and increases superoxide production in cultured endothelial cells [6,9]. In addition, we and others have demonstrated a change in HDL protein cargo in the presence of chronic inflammatory disease

Abbreviations: HDL, high density lipoprotein; CV, cardiovascular; CAD, coronary artery disease; NO, nitric oxide; PD, periodontitis; HAECs, human aortic endothelial cells; TNFα, tumor necrosis factor-alpha; ApoB, apolipoprotein B; SAA, serum amyloid alpha; CRP, C-reactive protein; ESR, electron spin resonance; VCAM-1, vascular cell adhesion protein 1.

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and coronary artery disease (CAD) [10,11]. These functional changes in HDL may explain the lack of benefit from agents which elevate plasma HDL levels reported in recent large-scale clinical trials.

Our group has extensively characterized a novel human inflammatory model in patients with periodontitis (PD) [12]. Using this model, we examined whether chronic and transient changes in inflammation associate with HDL functions including cholesterol efflux properties.

2. Methods and materials

2.1. Study population and protocol

Consecutive patients, referred to the Periodontology Unit of the UCL Eastman Dental Institute and Hospital, were invited to participate in a longitudinal study. Only individuals presenting with severe PD, defined as the presence of probing pocket depths of >6 mm and marginal alveolar bone loss of >30% affecting >50% of teeth were recruited as previously described [12]. Patients who had other systemic illness, a history of acute or chronic infection (assessed by the examining clinician), or who were receiving antibiotics or other regular CV medication were excluded.

2.1.1. Study 1

Vascular properties of HDL from 26 patients with severe PD were compared to 26 healthy control subjects selected with comparable age and gender characteristics, and consecutively enrolled by the Blood Donation Service of the University Hospital Zurich, without any CV risk factors (by history, clinical examination, and laboratory tests) or accompanying disorders.

2.1.2. Study 2

26 PD patients underwent treatment with a well characterized inflammatory response [13]. HDL function was studied at baseline, after 24 h and at 6 months following treatment. These time-points were selected as they had previously been shown to correspond to the peak and resolution of the systemic inflammatory response.

The study was approved by the joint Eastman and University College Hospitals ethics committee and all patients provided informed consent.

2.2. Anthropometric and biochemical measurements

Anthropometric measurements were recorded and body mass index (BMI [kg/m^2]) was calculated from weight and height. Blood pressure was measured in triplicate (HEM-705CP, Omron) and the average of the readings was recorded. Blood samples were drawn and processed after an overnight fast and serum and plasma samples were stored at −70 °C for subsequent analysis. Full blood count, lipid and glucose level measurements were made with standard biochemistry assays and C-reactive protein (CRP) was measured with an immunoturbidimetric, high-sensitivity assay (Tina-quant CRP immunoturbidimetric assay performed on a Cobas Integra analyzer, Roche Diagnostics). Inter and intra assay coefficients of variations for all assays were <5%.

2.3. HDL measurements

2.3.1. HDL isolation

HDL was isolated by sequential ultracentrifugation (d = 1.063–1.21 g/mL) using solid potassium bromide for density adjustment [14].

2.3.2. Electron spin resonance (ESR) measurement of NO bioavailability

The effect of HDL from patients and controls (50 μg/mL; 60 min, 37 °C) on endothelial NO production ((human aortic endothelial cells) HAECs; passage 4–7; Lonza Bio Science) was examined by continuous-wave electron spin resonance (ESR) spectroscopy using the spin-probe colloid Fe(DETC)2 (Noxygen). ESR spectra of samples frozen in liquid nitrogen were recorded on a Bruker e-scan spectrometer (Bruker BioSpin) with the following instrumental settings: center field (B0) 3455 G; sweep width 80 G; microwave power 39.72 mW; modulation amplitude 10.34 G; sweep time 10.49 s; number of scans 10 [6,15]. Our group has reported has coefficients of variation for intraassay and interassay variability of these measurements as 1.92% and 1.74%, respectively.

2.3.3. ESR measurement of superoxide production

The effect of HDL on endothelial cell superoxide production was compared to controls in un-stimulated and tumor necrosis factor (TNF)α-stimulated (5 ng/mL, R&D Systems) HAECs by ESR spectroscopy. Briefly, HAECs were incubated with HDL (50 μg/mL, 60 min, 37 °C), with or without TNFα and re-suspended in Krebs-Hepes buffer (pH 7,4; Noxygen) containing diethyldithiocarbamic acid sodium salt (5 μM, Noxygen) and deferoxamine methanesulfonate salt (25 μM, Noxygen). ESR spectra were recorded after addition of the spin probe 1-hydroxy-3-methoxycarbonyl-2,2,5,5-tetramethylpyrrolidine (CMH); Noxygen; final concentration (200 μM) using a Bruker e-scan spectrometer (Bruker BioSpin). The ESR instrumental settings were as follows: center field (B0) 3495 G; field sweep width 10 G; microwave frequency 9.75 GHz; microwave power 19.91 mW; magnetic field modulation frequency 86.00 kHz; modulation amplitude 2.60 G; conversion time 10.24 msec; number of x-scans 1020 [6]. The coefficients of variation for intra-assay and interassay variability of these measurements were 5.1% and 12.9%, respectively.

2.3.4. Cholesterol efflux capacity of HDL

HDL for measurements of efflux capacity was extracted from serum by ApoB depletion. Briefly, whole serum was incubated for 20 min with a 20% polyethylene glycol (PEG) solution (20% PEG 8000 (sigma P2139) in 200 mM glycine (sigma G8898, pH = 10)). Samples were centrifuged at 1900 G, and the supernatant was collected and stored at 4 °C.

Cholesterol efflux capacity was quantified according to a method previously reported [16]. J774 cells were radiolabeled for 24 h in a medium containing 2 μCi of [^{3}H]-cholesterol per mL. Addition of 0.3 mM 8-(4-chlorophenylthio)-cyclic AMP for 6 h up-regulated expression of ABCA1. An efflux medium containing 2.8% apolipoprotein B-depleted serum from each individual was added for 4 h. To prevent cholesterol esterification during labeling, equilibration and flux, 2 μg per mL of CP113,818, a acyl-coenzyme A:cholesterol acyltransferase inhibitor was added to all mediums. Efflux capacity was quantified using liquid scintillation to measure radioactive cholesterol effluxed from the cells (medium + intracellular lipids). All assays were performed in duplicate and the final average value normalized against a baseline control for statistical analyses between time-points.

2.3.5. Serum assay of HDL function

Serum paraoxonase activity was measured by UV spectrophotometry in a 96-well plate format using paraoxon (Sigma-Aldrich, St Louis, Missouri). Briefly, 50 μg/mL HDL was diluted in a reaction mixture containing 10 mM Tris hydrochloride (pH 8.0), 1 M sodium chloride and 2 Mm calcium chloride. At 24 °C 1.5 mM paraoxon was added to initiate the reaction, and the increase in absorbance at 405 nm was recorded over 30 min. An extinction coefficient (at 405 nm and 24 °C) of 17 000 $M^{-1}\cdot cm^{-1}$ was used to calculate units of paraoxonase activity [17]. The coefficients of variation for intra-assay and inter-assay variability of these measurements were 7.2% and 13.1%, respectively.

3. Statistics

All measures are reported as mean (SD) or median [IQR] for those not normally distributed. Normal distribution was assessed using the Kolmogorov–Smirnov test. Comparisons between the HDL measurements and inflammatory markers were performed using the Pearson correlation coefficient for normally distributed data and the Spearman Rank Correlation method for non-normally distributed data. Comparisons