Journal of the American College of Cardiology
Published by Elsevier Inc.
Vol. 60, No. 23, 2012
ISSN 0735-1097/$36.00
http://dx.doi.org/10.1016/j.jacc.2012.09.013
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Dysfunctional High-Density Lipoprotein in Patients on Chronic Hemodialysis

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Objectives This study examined the functionality of high-density lipoprotein (HDL) in individuals with end-stage renal disease on dialysis (ESRD-HD).

Background The high rate of cardiovascular disease (CVD) in chronic kidney disease is not explained by standard risk factors, especially in patients with ESRD-HD who appear resistant to benefits of statin therapy. HDL is antiatherogenic because it extracts tissue cholesterol and reduces inflammation.

Methods Cellular cholesterol efflux and inflammatory response were assessed in macrophages exposed to HDL of patients with ESRD-HD or controls.

Results HDL from patients with ESRD-HD was dramatically less effective than normal HDL in accepting cholesterol from macrophages (median 6.9%; interquartile range [IQR]: 1.4% to 10.2%) versus control (median 14.9%; IQR: 9.8% to 17.8%; $p < 0.001$). The profound efflux impairment was also seen in patients with ESRD-HD and diabetes compared with patients with diabetes without renal disease (median 8.1%; IQR: 3.3% to 12.9%) versus control (median 13.6%; IQR: 11.0% to 15.9%; $p = 0.009$). In vitro activation of cellular cholesterol transporters increased cholesterol efflux to both normal and uremic HDL. HDL of patients with ESRD-HD had reduced antichemotactic ability and increased macrophage cytokine response (tumor necrosis factor-alpha, interleukin-6, and interleukin-1-beta). HDL of patients with ESRD-HD on statin therapy had reduced inflammatory response while maintaining impaired cholesterol acceptor function. Interestingly, impaired HDL-mediated efflux did not correlate with circulating C-reactive protein levels or cellular inflammatory response.

Conclusions These findings suggest that abnormal HDL capacity to mediate cholesterol efflux is a key driver of excess CVD in patients on chronic hemodialysis and may explain why statins have limited effect to decrease CV events. The findings also suggest cellular cholesterol transporters as potential therapeutic targets to decrease CV risk in this population. (J Am Coll Cardiol 2012;60:2372–9) © 2012 by the American College of Cardiology Foundation

Chronic kidney disease (CKD) dramatically increases cardiovascular disease (CVD) risk, which escalates with declining kidney function and is maximal in patients with end-stage renal disease on chronic hemodialysis (ESRD-HD) (1–3). Although increased CV mortality in patients with ESRD-HD is attributable to many causes, death from atherosclerotic coronary artery disease is persistently higher than that in the general population, even after adjustments for traditional CVD risk factors (2–6). Patients with ESRD-HD are also unique in their diminished benefit from statin interventions that are effective in reducing CVD risk in other high-risk patients (7–9). The recent SHARP (Study of Heart and Renal Protection) trial reported decreased

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numbers of CV events with aggressive cholesterol lowering using simvastatin and ezetimibe, in patients with CKD (9,10). Results were overall positive, but the difference in the ESRD-HD subgroup was too small to show statistical significance. The lack of benefit of aggressive lipid-lowering

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Manuscript received April 9, 2012; revised manuscript received September 5, 2012, accepted September 5, 2012.

therapy in ESRD suggests other mechanisms, such as low levels of high-density lipoprotein (HDL). HDL has generated considerable interest as a target for interventions to decrease atherosclerotic burden (11–13). One key function of HDL, namely macrophage cholesterol efflux capacity (14–18), was inversely associated with measures of carotid and coronary atherosclerosis in a non-CKD population, independent of HDL concentration (18).

Interestingly, Holzer et al. (14) recently reported reduced efflux ability of HDL in patients with ESRD-HD compared with HDL from controls without kidney disease. Although intriguing, these data did not examine whether coexisting comorbidities such as diabetes, obesity, underlying CVD, and certain demographic characteristics of patients with ESRD-HD (e.g., age, sex, race, underlying CKD) contributed to the observed differences. It is critically important to determine the mechanisms by which abnormalities in HDL function predispose patients with CKD to increased atherosclerotic burden to develop rational therapeutic strategies. In this study, we evaluated the ability of HDL from patients with ESRD-HD to elicit cholesterol efflux and modulate inflammatory responses in macrophages and compared this with HDL from individuals with normal kidney function with similar coexisting clinical and demographic characteristics that influence development of atherosclerotic CVD (19).

Methods

Participants and study design. Adult patients with ESRD undergoing maintenance hemodialysis at Vanderbilt University Medical Center (VUMC) for more than 3 months were studied (n = 29). Control participants (n = 28) with normal renal function who did not differ from the patients in age, sex, race, presence of diabetes mellitus, history of CVD (angina, myocardial infarction, stroke/transient ischemic attack), and use of statin and angiotensin-converting enzyme inhibitors (ACEIs)/angiotensin receptors blockers (ARBs) were recruited from the general medicine outpatient clinic at VUMC. Exclusion criteria included pregnancy and current smoking. The study protocols were approved by the institutional review board at VUMC, and all participants gave informed consent.

Assessment of plasma lipid profile and C-reactive protein and isolation of lipoproteins. Blood was taken after an overnight fast (prior to hemodialysis) in K2 ethylenediamine tetraacetic acid–containing tubes and centrifuged at 1,700 *g* for 15 min. Plasma levels of total cholesterol, triglycerides, and HDL were measured enzymatically (Cliniqa, San Marcos, California). High-sensitivity C-reactive protein (hsCRP) was measured using a high-sensitivity immunoturbidimetric assay (Roche Modular Systems, Indianapolis, Indiana) (20). The apolipoprotein B (apoB)–containing fraction (d = 1.006 to 1.063 g/ml) and HDL fraction (d = 1.063 to 1.21 g/ml) were isolated from fresh plasma by sequential density ultracentrifugation after density adjustment with potassium bromide (21). The purity of the isolation was checked by the QuickGel lipoprotein electrophoresis system (Helena Laboratories, Beaumont, Texas).

Macrophage assays of cholesterol uptake with apoB fraction and cholesterol efflux with HDL. THP-1 cells (American Type Culture Collection, Manassas, Virginia) were plated onto 35-mm wells and differentiated into macrophages by RPMI containing 10% fetal bovine serum and 50 ng/ml phorbol 12-myristate 13-acetate. For uptake studies, THP-1 macrophages were exposed to the apoB fraction (50 μg/ml) from patients with ESRD-HD or controls for 24 h. For efflux studies, THP-1 macrophages were cholesterol enriched with acetylated low-density lipoprotein (LDL) (100 μg/ml, Intracel, Frederick, Maryland) and exposed to HDL (50 μg/ml) and lipopolysaccharide (LPS) (50 ng/ml, Sigma-Aldrich Co., St. Louis, Missouri) for 24 h. In some studies, liver X receptor (LXR) agonist T0901317 (1 μM, Sigma-Aldrich) was added to activate ATP-binding cassette (ABC) transporters. Cellular cholesterol content was measured by gas chromatography (21,22). Cholesterol efflux was determined as the percent decrease in cellular cholesterol content at baseline versus after incubation with HDL (15,23). Cell proteins were solubilized by addition of 0.1 N NaOH and protein content was measured by bicinchoninic acid assay.

Abbreviations and Acronyms

ABC = ATP-binding cassette

apoB = apolipoprotein B

CKD = chronic kidney disease

CVD = cardiovascular disease

ESRD-HD = end-stage renal disease on chronic hemodialysis

HDL = high-density lipoprotein

hsCRP = high-sensitivity C-reactive protein

IL = interleukin

LDL = low-density lipoprotein

LXR = liver X receptor

MCP = monocyte chemotactic protein

TNF = tumor necrosis factor

Macrophage chemotaxis assay and inflammatory reaction with HDL. Macrophage migrating activity used THP-1 cells exposed to HDL from patients with ESRD-HD or controls. The studies were performed in a microchemotaxis chamber in which the upper and lower compartments are separated by an uncoated polycarbonate filter (Neuroprobe, Gaithersburg, Maryland). THP-1 cells (5×10^5/well) were exposed to HDL (50 μg/ml) for 1 h and then added to the upper compartment. Monocyte chemotactic protein (MCP)-1 0.1 μg/ml (Preprotech, Rocky Hill, New Jersey) was added to the lower compartment. Filters were fixed in methanol and stained with 1% crystal violet, and the migrated cells were counted under the microscope. Duplicate wells were used for each experimental condition, and 5 fields (×40) were counted for each well. The anti-inflammatory function of HDL was measured as the cytokine response in LPS-activated macrophages. THP-1 macrophages were exposed to HDL (50 μg/ml) and LPS (50 ng/ml) for 4 to 24 h. Total RNA was extracted from cells with the RNeasy mini kit (Qiagen, Valencia, California). Quantification of human interleukin (IL)-1-beta, IL-6,

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