JOURNAL OF THE AMERICAN COLLEGE OF CARDIOLOGY
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PUBLISHED BY ELSEVIER INC.

VOL. 66, NO. 13, 2015
ISSN 0735-1097/$36.00
http://dx.doi.org/10.1016/j.jacc.2015.07.057

Defects of High-Density Lipoproteins in Coronary Artery Disease Caused by Low Sphingosine-1-Phosphate Content

Correction by Sphingosine-1-Phosphate—Loading

Katherine Sattler, MD,* Markus Gräler, PhD,† Petra Keul, PhD,* Sarah Weske, MSc,* Christina-Maria Reimann, MSc,† Helena Jindrová, MSc,* Petra Kleinbongard, PhD,* Roger Sabbadini, PhD,‡ Martina Bröcker-Preuss, MD,§ Raimund Erbel, MD,‖ Gerd Heusch, MD,* Bodo Levkau, MD*

ABSTRACT

BACKGROUND Sphingosine-1-phosphate (S1P) is a constituent of high-density lipoproteins (HDL) that contributes to their beneficial effects. We have shown decreased HDL-S1P in coronary artery disease (CAD) but its functional relevance remains unclear.

OBJECTIVES This study investigated the functional consequences of reduced HDL-S1P content in CAD and tested if increasing it may improve or restore HDL function.

METHODS Human HDL from healthy and CAD subjects, as well as mouse HDL, were isolated by ultracentrifugation. HDL-S1P-dependent activation of cell-signaling pathways and induction of vasodilation were examined in vitro and in isolated arteries using native and S1P-loaded HDL, S1P receptor antagonists, and S1P-blocking antibodies.

RESULTS HDL-S1P-dependent signaling was clearly impaired and S1P content reduced in CAD-HDL as compared to healthy HDL. Both healthy and CAD-HDL could be efficiently and equally well loaded with S1P from cellular donors and plasma. S1P-loading greatly improved HDL signaling and vasodilatory potential in pre-contracted arteries and completely corrected the defects inherent to CAD-HDL. HDL-S1P content and uptake was reduced by oxidation and was lower in HDL_3 than HDL_2. Loading with S1P in vitro and in vivo fully replenished the virtually absent S1P content of apolipoprotein M-deficient HDL and restored their defective signaling. Infusion of erythrocyte-associated C17-S1P in mice led to its rapid and complete uptake by HDL providing a means to directly S1P-load HDL in vivo.

CONCLUSIONS Reduced HDL-S1P content contributes to HDL dysfunction in CAD. It can be efficiently increased by S1P-loading in vitro and in vivo, providing a novel approach to correcting HDL dysfunction in CAD. (J Am Coll Cardiol 2015;66:1470–85) © 2015 by the American College of Cardiology Foundation.

From the *Institute for Pathophysiology, West German Heart and Vascular Center, University Hospital Essen, Essen, Germany; †Department of Anesthesiology and Intensive Care Medicine, Center for Sepsis Control and Care, and Center for Molecular Biomedicine, University Hospital Jena, Jena, Germany; ‡Lpath Inc., San Diego, California; §Department of Clinical Chemistry, University Duisburg-Essen, Essen, Germany; and the ‖Clinic of Cardiology, West German Heart and Vascular Center, University Hospital Essen, Essen, Germany. Supported by the Deutsche Forschungsgemeinschaft, Bonn, Germany (Schwerpunktprogramm Sphingolipids-Signals and Disease SPP 1267, project LE940/4-2; GRK 2098, projects 9, 10, and SFB 656 Münster, project A6), as well as the Hans und Gertie Fischer Stiftung. Dr. Sabbadini is a founder and shareholder of Lpath, Inc. All other authors have reported that they have no relationships relevant to the contents of this paper to disclose.
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Manuscript received May 31, 2015; revised manuscript received July 15, 2015, accepted July 22, 2015.

High-density lipoproteins (HDLs) confer protection against the development of atherosclerosis (1). In addition to their crucial role in reverse cholesterol transport, HDLs have several reverse cholesterol transport–independent properties that potentially contribute to atheroprotection (2,3). Patients with clinically manifest atherosclerosis not only have low plasma HDL cholesterol levels but their HDLs are dysfunctional and harbor harmful metabolites (4). However, the reasons for this dysfunction and the biochemical, structural, and molecular correlates of HDL driving it are largely unknown. Among the most frequently proposed causes are oxidative modifications of HDL proteins and lipids (5,6).

In previous work, we and others have shown that HDLs contain sphingosine-1-phosphate (S1P) that contributes to several beneficial HDL effects, such as nitric oxide-dependent vasodilation and cardioprotection (3,7–11). Recently, we have shown that HDLs from patients with coronary artery disease (CAD) contain considerably lower amounts of S1P than healthy HDLs (12). However, it remains unknown whether this reduced S1P content translates into HDL dysfunction or, conversely, if increasing it may improve or restore CAD-HDL function.

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In the present study, we investigated the functional consequences of low S1P in CAD-HDL. We compared activation of signaling pathways by healthy HDLs and CAD-HDLs and have designed strategies to increase HDL-S1P content in vitro and in vivo to improve HDL function. We also examined whether CAD-HDL signaling may be impaired due to low S1P content and if this can be corrected by S1P-loading.

METHODS

A detailed description of all experimental procedures is provided in the Online Appendix.

In brief, venous blood was drawn from healthy human subjects (n = 70) and patients with stable CAD (n = 64) recruited consecutively to expand the groups used in our previous studies of S1P in CAD-HDL (12). The study was approved by the ethics committee of the University Hospital Essen and complies with the Declaration of Helsinki. Written informed consent was obtained from each participant. Demographic and clinical data are provided in Online Table 1. HDLs were isolated by sequential density gradient ultracentrifugation as described (12) from pooled plasma (mice) or individual samples (humans).

S1P was measured in plasma and HDLs using liquid chromatography tandem mass spectrometry (LCMS) (12) by blinded investigators.

HDLs were loaded with S1P either by incubation with sphingosine-laden human erythrocytes (10 μmol/l sphingosine for 1 h) or by adding 6 nmol S1P (in vitro studies) or 3 pmol S1P (vasodilation studies) to 0.1 mg HDL.

C17-laden mouse erythrocytes (10 μmol/l C17-S1P for 1 h) were extensively washed, diluted to a hematocrit of 0.5, and injected intravenously (150 μl).

STATISTICAL ANALYSIS. Data are expressed by mean ± SD or median (range) for continuous variables and frequency count and percentage for qualitative variables, respectively. Groups were compared by unpaired or paired Student *t* test, Mann-Whitney *U* test, or chi-square test. Statistical significance was assumed for $p < 0.05$.

ABBREVIATIONS AND ACRONYMS

apo = apolipoprotein
CAD = coronary artery disease
CHO = Chinese hamster ovarian cells
DOP = 4-deoxypyridoxine
eNOS = endothelial nitric oxide synthase
ERK1/2 = extracellular signal-regulated kinases 1/2
HDL = high-density lipoprotein
HNF 1A = hepatocyte nuclear factor 1 alpha
HUVEC = human umbilical vein endothelial cell
S1P = sphingosine-1-phosphate

RESULTS

IMPAIRED CAD-HDL SIGNALING DUE TO LOWER S1P CONTENT. We have previously shown that CAD-HDLs contain lower S1P concentrations than HDLs from healthy individuals in a total of 95 healthy subjects and 85 patients with stable CAD (12). To test whether this translates into impaired S1P-dependent HDL signaling, we compared the effectiveness of healthy HDL and CAD-HDL to activate 3 important intracellular signaling cascades in human umbilical vein endothelial cells (HUVEC): the extracellular signal-regulated kinases 1 and 2 (ERK1/2) mitogen activated protein kinases, the Akt pathway, and the endothelial nitric oxide synthase (eNOS). We used Western blotting to detect the active phosphorylation sites of ERK1/2 (Thr202/Tyr204), Akt (Ser473), and eNOS (Ser1177). HDL preparations from 14 healthy subjects and 9 patients with stable CAD (Online Table 2) were chosen randomly and compared. As measured by LCMS, CAD-HDL contained 4 to 5 times less S1P than healthy HDL (45.06 ± 6.74 pmol/mg of HDL protein vs. 273.78 ± 15.01 pmol/mg; $p < 0.05$). Using this experimental setup, we observed that CAD-HDLs were much less efficient than healthy HDLs in activating all 3 kinases both in magnitude and duration of the evoked signaling response when used at the same protein concentration (**Figure 1A**).

To test if the observed differences can be attributed to the differences in HDL-S1P content, we established a cellular system in which S1P-dependent