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SCIENCE DIRECT

Biochimica et Biophysica Acta 1733 (2005) 76–89

BIOCHIMICA ET BIOPHYSICA ACTA
BBA

http://www.elsevier.com/locate/bba

Regular paper

Phosphatidylcholine-rich acceptors, but not native HDL or its apolipoproteins, mobilize cholesterol from cholesterol-rich insoluble components of human atherosclerotic plaques

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Received 12 June 2003; received in revised form 21 January 2004; accepted 2 December 2004
Available online 23 December 2004

Abstract

To examine the potential of high density lipoproteins (HDL) to ameliorate atherosclerotic plaques in vivo, we examined the ability of native HDL, lipid-free HDL apolipoproteins (apo HDL), cholesterol-free discoidal reconstituted HDL (R-HDL) comprised of apo HDL and phosphatidylcholine (PC) and PC liposomes to release cholesterol from cholesterol-rich insoluble components of plaques (ICP) isolated from atherosclerotic human aorta. Isolated ICP had a free cholesterol (FC) to phospholipid (PL) mass ratio (0.8–3.1) and a sphingomyelin (SPM) to PC mass ratio (1.2–4.2) that exceeded those of plasma membranes of cultured cells. Suprisingly, native HDL and its apolipoproteins were not able to release cholesterol from ICP. However, R-HDL and PC liposomes were effectively released cholesterol from ICP. The release of ICP cholesterol by R-HDL was dose-dependent and accompanied by the transfer of >8× more PC in the reverse direction (i.e., from R-HDL to ICP), resulting in a marked enrichment of ICP with PC. Compared to R-HDL, PC liposomes were significantly less effective in releasing cholesterol from ICP but were somewhat more effective in enriching ICP with PC. Native HDL was minimally effective in enriching ICP with PC, but became effective after prior in vitro enrichment of HDL with PC from multilamellar PC liposomes. The enrichment of ICP with PC resulted in the dissolution of cholesterol crystals on ICP and allowed the removal of ICP cholesterol by apo HDL and plasma. Our study revealed that the removal of cholesterol from ICP in vivo will be possible through a change in the level, composition, and physical state of ICP lipids mediated by PC-enriched HDL.

Keywords: Atherosclerotic plaque; Cholesterol; Cholesterol crystal; Phospholipid; Sphingomyelin; Phosphatidylcholine; High density lipoprotein; Plaque regression

Abbreviations: apo, apolipoproteins; CE, cholesterylester; CETP, cholesterylester transfer proteins; DMPC, dimyristoyl phosphatidylcholine; FC, free cholesterol; ICP, insoluble component of plaques; LCAT, lecithin:cholesterol acyltransferase; PC, phosphatidylcholine; PL, phospholipids; RBC, red blood cells; RCT, reverse cholesterol transport; R-HDL, reconstituted HDL; SPM, sphingomyelin; TBS, Tris-buffered saline; TLC, thin-layer chromatography

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doi:10.1016/j.bbalip.2004.12.002

1. Introduction

In numerous epidemiological and clinical studies, plasma levels of HDL and apo A-I correlated inversely with the incidence of coronary artery disease (CAD) [1–3]. This protective effect is usually attributed to HDL's role in removing cholesterol from atherosclerotic lesions and transporting it to the liver, a process called reverse cholesterol transport (RCT) [4]. In vitro cell culture studies have shown that native HDL or its lipid-free apolipoproteins are able to promote the efflux of cellular cholesterol via a non-specific aqueous diffusion process [5,6], mediation by specific scavenger receptor class B type I (SR-BI) receptors on cell surfaces [7,8], and/or microsolubilization of plasma membrane PL and cholesterol, with the participation of ATP-binding cassette transporter A1 (ABCA1) [9–11]. Cholesterol deposits are an essential characteristic of human atherosclerotic lesions, but the deposit of cholesterol on atherosclerotic lesions differs from that on membranes of cultured cells. In early atherosclerotic lesions, cholesterol accumulates on cells, as intracellular lipid droplets in foam cells, and as extracellular lipoprotein- and liposome-like particles [12–18]. As lesions progress, foam cells die, leading to an increasingly necrotic plaque core [12,13,17,18]. Thus, plaque cholesterol composition is complex, containing living and dead cells, cellular debris, and extracellular particles including crystalline cholesterol [12–18]. It is not clear whether HDL and its apolipoproteins can release cholesterol in advanced atherosclerotic lesions containing dead foam cells and extracellular cholesterol deposits by the same mechanisms involved in the release of cholesterol from cultured cells. It is probable that SR-B1 receptor and ABCA1 transporter may not have an important role in removing cholesterol from advanced atherosclerotic lesions containing dead foam cells and/or extracellular deposits because the activities of the SR-B1 receptor and ABCA1 transporter would not be preserved.

In humans and animal models, advanced atherosclerotic lesions contain crystallized cholesterol and are highly enriched in FC and SPM [12,15,19]. SPM-enrichment stabilizes cholesterol on cell membranes, and high FC levels may protect the dissolution of lipids in the advanced lesions by HDL and apo A-I [16]. Further, the turnover of cholesterol on atherosclerotic plaques in humans is much slower than that in other tissues due to the change in their physical state [20,21], suggesting that the exchange rate of cholesterol between plaques and blood is low. For these reasons, the mechanisms and kinetics of HDL- and apolipoprotein-mediated cholesterol removal from cultured cells may not reflect those of HDL-mediated cholesterol removal from advanced atherosclerotic lesions. Indeed, early studies suggested that a fundamentally different process occurs in the intima, as pure cholesterol crystals incubated with HDL form liposomes containing PL derived from HDL [22,23]. Here, we test this possibility by examining the potencies of native HDL, cholesterol-free reconstituted HDL (R-HDL), apo HDL, and fresh plasma to solubilize cholesterol on ICP that we have previously isolated and partially characterized [24]. We find that PC-enriched R-HDL and native HDL can dissolve crystalline cholesterol deposits.

2. Method

2.1. Reagents

Egg PC, egg SPM, dimiristoylphosphatidylcholine (DMPC), and FC were purchased from Avanti Polar Lipid, Birmingham, Alabama. Bacterial (*Bacilus cereus*) sphingomyelinase was purchased from Sigma Chemical, St. Louis, MO. Enzymatic assay reagents for cholesterol, FC and PL and an immunoassay kit for apo A-I were purchased from Wako Diagnostic, Richmond, VA.

2.2. Preparation of ICP

Human atherosclerotic aortas (8 to 30 h post-mortem), evaluated by a pathologist, were obtained from the Tissue Procurement Facility at the University of Alabama Medical Center. The Institutional Review Board at the University of Alabama approved the use of human tissues in this study. Tissues were rinsed 5 times with Tris-buffered saline (TBS) (0.15 M NaCl–0.01 M Tris, pH 7.4) containing 0.1% EDTA, 20 μM butylated hydroxytoluene, and proteinase inhibitor cocktails. Aortic intima containing grossly visible plaques (i.e. yellow-white to golden yellow streaks and/or patches) was stripped from the media, washed 4× with TBS, and minced finely with scissors. After large tissue fragments were pelleted by a low speed centrifugation (700×*g* for 10 min), they were gently homogenized, and the homogenates were centrifuged again. ICP in the pooled upper supernatant fractions were fractionated by a modification of a density gradient ultracentrifugation method described previously [25]. Briefly, the density of the supernatant was adjusted to d=1.21 g/ml and placed at the bottom of ultracentrifuge tubes (Beckman SW 41 rotor tubes) containing 4 ml TBS layered on top of a 4 ml KBr solution (d=1.12 g/ml) [26]. After centrifugation at 40,000 rpm for 3 h, flocculent lipid particles banded at d=1.02 g/ml to d=1.07 g/ml density ranges (lower- to upper-middle portion) of the density gradient tube. Greater than 65% of the cholesterol in the density gradient tube was in the d=1.02–1.07 g/ml density fraction. Following the removal of KBr from pooled middle fractions by dialysis against TBS at 4 °C for 16 h and subsequent centrifugation at 10,000 rpm for 15 min in a microcentrifuge, ICP were recovered from the bottom of the centrifuge tubes. Isolated ICP were then washed with TBS until little or no cholesterol was released. Most (>85%) of cholesterol in the dialyzed d=1.02–1.07 g/ml density fraction was associated with ICP. Few or no ICP was recoverable in the d=1.02–1.07 g/ml