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A Novel Atheroprotective Mechanism for High Density Lipoprotein: Attenuation of Endothelial Cell Inflammation

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Objective:

The National Cholesterol Education Program (NCEP) encourages the measurement of High Density Lipoprotein (HDL) since HDL augments reverse cholesterol transport and thus protects against atherosclerosis. Recent studies suggest that HDL may have additional benefits distinct from cholesterol trafficking. This study proposes a novel signaling pathway which HDL activates to prevent inflammation of human endothelial cells, an early stage of atherosclerosis. We investigated this phenomenon *in vitro* in a human endothelial cell culture system, and *in vivo* using a gene-knockout animal which was previously described to be plasma-deficient in HDL.

Methods:

Human aortic endothelial cells (HAEC) growing in culture were pretreated with the human HDL-3 sub-fraction, isolated by ultracentrifugation (0.5 to 50 mg/dL) or purified apolipoproteins from HDL: A-I, A-II, C-I, or E (10^{-5} to 10^{-2} mg/mL), then stimulated with thrombin. Concentrations of von Willebrand Factor (vWF) released from thrombin stimulated cells was quantified by ELISA. Activated intracellular signaling cascades downstream from the HDL receptor (SR-BI) were assessed by Western blotting. Specific signaling pathways were suppressed using commercially available antagonists. SR-BI was also blocked using antibodies or the chemical inhibitor, maleylated albumin. A human promyelocytic leukemia cell line (HL-60) was loaded with a fluorescent dye and added to HAEC previously stimulated with thrombin. Subsequent cell adhesion was visualized by fluorescent microscopy, and quantified using a fluorimeter. A fluorescent dye was injected into wild-type or apoA-I gene knockout (apoA-I^{-/-}) mice retro-orbitally to label leukocytes, mesenteric venules were isolated *in situ*, and fluorescent leukocyte adhesion and rolling velocity *in vivo* were assessed by intravital fluorescent microscopy.

Results:

Physiologic concentrations of HDL-3 (50 mg/dL) inhibit vWF release from HAEC ($p=0.007$) and this effect is both dose- and time-dependent. ApoA-I 10^{-5} mg/mL purified from human HDL inhibits vWF release from HAEC ($p=0.03$), and this effect is dose-dependent. A 1000-fold higher concentration of apoA-II was required for a similar inhibition of vWF release from HAEC ($p=0.011$), and a 10-fold higher concentration of apoC-I or apoE are needed for similar inhibition of vWF release from HAEC ($p=0.004$ and $p=0.001$, respectively). Basal cell viability is enhanced in the absence of thrombin by pre-treatment of HAEC with 10^{-4} mg/mL apoA-I ($p=0.002$). The inhibition of vWF release from HAEC by apoA-I is relieved by prior treatment of HAEC with a specific protein kinase C (PKC) inhibitor, but not by inhibitors of the classical MAP Kinase (ERK-1/2) or the endothelial nitric oxide synthase (eNOS) pathways. Treatment of HAEC with thrombin enhances HL-60 cell surface adhesion by three-fold, highly suggestive of inflammation, and prior treatment with apoA-I attenuates HL-60 adhesion by 64%. Leukocyte adhesion and rolling velocity are significantly lower in apoA-I^{-/-} vs. WT mice (54 ± 8 $\mu\text{m}/\text{ms}$ vs. 72 ± 14 $\mu\text{m}/\text{ms}$, $p=0.003$) at baseline, implying a propensity for inflammation.

Conclusion:

ApoA-I, the major HDL apolipoprotein, prevents vWF release from endothelial cells and suppresses endothelial-to-leukocyte adhesion, thus displaying novel anticoagulative and antiinflammatory properties. We propose that HDL-associated apoA-I prevents endothelial cell inflammation by targeted activation of intracellular PKC.