Volume 7, issue 3, June 2006

ISSN 1567-5688

ELSEVIER

ATHEROSCLEROSIS

SUPPLEMENTS

OFFICIAL JOURNAL OF THE EUROPEAN ATHEROSCLEROSIS SOCIETY

AFFILIATED WITH THE INTERNATIONAL ATHEROSCLEROSIS SOCIETY AND THE SOCIETY OF ATHEROSCLEROSIS IMAGING AND PREVENTION

ABSTRACTS
XIV International Symposium on Atherosclerosis
Rome, Italy, June 18-22, 2006

Tuesday, June 20, 2006: Workshop

Tu-W16 HDL FUNCTIONS

Tu-W16:1 HDL AND THE INHIBITION OF INFLAMMATION IN VIVO

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Objective: *In vitro* studies have established that high density lipoproteins (HDL) from human plasma, as well as discoidal reconstituted HDL (rHDL) that contain apolipoprotein (apo) A-I, inhibit the cytokine-induced expression of vascular cell adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1) and E-selectin in cultured human umbilical vein endothelial cells [1]. More recently, we have shown that three serial infusions of discoidal rHDL or lipid-free apoA-I administered prior to, at the time of and following an inflammatory stimulus inhibit acute inflammation *in vivo* [2]. The objectives of the present *in vivo* studies are to determine (i) if a single, low dose infusion of lipid-free apoA-I or discoidal rHDL inhibits inflammation when administered prior to or at the time of the inflammatory stimulus, (ii) if a single dose of lipid-free apoA-I inhibits inflammation when administered after the inflammatory stimulus and (iii) if non-enzymatic glycation of apoA-I, as occurs in diabetic subjects, affects the anti-inflammatory properties of HDL.

Methods: Lipid-free apoA-I was isolated from human plasma by standard techniques. Discoidal rHDL containing phosphatidylcholine and apoA-I were prepared by the cholate dialysis method. Lipid-free apoA-I and the apoA-I in the discoidal rHDL were non-enzymatically glycated by incubation with methylglyoxal. Non-occlusive, silastic collars were inserted around the carotid arteries of normocholesterolemic New Zealand White rabbits. The animals received a single infusion of either saline, lipid-free apoA-I, or discoidal rHDL either 24 prior to, at the time of, or at 3 or 9 h after collar insertion. Other animals received glycated lipid-free apoA-I or discoidal rHDL that contained glycated apoA-I 24 prior to collar insertion. The animals were sacrificed 24 h after collar insertion and the carotid arteries were removed for analysis. Neutrophil recruitment into the intima-media and endothelial expression of vascular cell adhesion molecule-1 (VCAM-1), and intercellular adhesion molecule-1 (ICAM-1) were quanititated by immunohistochemistry.

Results: Insertion of a periarterial collar induced substantial endothelial expression of VCAM-1 and ICAM-1, as well as extensive infiltration of neutrophils into the artery wall of the saline-infused animals. When the animals received a single infusion of either lipid-free apoA-I or discoidal rHDL, at a dose of 8 mg apoA-I/kg either 24 h prior to or at the time of collar insertion, infiltration of neutrophils into the artery wall and endothelial expression of VCAM-1 and ICAM-1 decreased to baseline levels. Neutrophil infiltration into the artery wall and endothelial expression of VCAM-1, but not ICAM-1 was still inhibited effectively when the animals were infused with lipid-free apoA-I at doses as low as 2 mg/kg. Comparable results were obtained when lipid-free was infused into the animals 3 h after collar insertion. Infusion of lipid-free apoA-I 9 h after collar insertion inhibited infiltration of neutrophils into the artery wall and endothelial expression of VCAM-1, but not ICAM-1. When the animals were infused with non-enzymatically glycated lipid-free apoA-I, or with discoidal rHDL in which the apoA-I was non-enzymatically glycated, the inhibition of neutrophil infiltration into the artery wall and adhesion molecule expression were both markedly attenuated.

Conclusions: A single infusion of lipid-free or lipid–associated apoA-I inhibits acute inflammation in rabbit carotid arteries, even when administered after an inflammatory stimulus. Glycated apoA-I, in either the lipid-free form, or as a constituent of discoidal rHDL inhibits inflammation much less effectively than unmodified apoA-I.

References

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Funding: The National Health and Medical Research Council of Australia.

Tu-W16:2 CHOLESTERYL ESTER TRANSFER PROTEIN (CETP) PRODUCTION BY MACROPHAGES PROMOTES ATHEROSCLEROTIC LESION DEVELOPMENT

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Objective: Inhibition of CETP is considered a promising new therapeutic strategy to increase HDL cholesterol levels and reduce atherosclerosis. Abundant amounts of CETP are found in macrophage-derived foam cells in the arterial wall, but its function in atherogenesis is unknown.

Methods: In order to investigate the function of macrophage CETP in atherosclerosis, LDL receptor knockout mice, which naturally lack CETP, were transplanted with bone marrow from CETP transgenic mice, which express the human CETP transgene under control of its natural promoter and major regulatory elements. Atherosclerotic lesion formation was induced by feeding a Western-type diet containing 0.25% cholesterol and 15% fat for 9 weeks.

Results: Macrophage CETP production induced a 1.8-fold ($p<0.01$) increase in atherosclerotic lesion development ($561\pm52\times10^3$ μm^2 in mice with macrophage CETP expression (n=9), as compared to $309\pm36\times10^3$ μm^2 in control mice (n=9)). The increase in lesion size, coincided with an increase in VLDL/LDL cholesterol from 993±177 mg/dL to 1761±167 mg/dL ($p<0.01$). Conversely, HDL cholesterol was decreased from 76±7 mg/dL to 53±7 mg/dL ($p<0.05$). Furthermore, the relative amount of the cholesterol-poor prebeta-HDL subclass was 3-fold ($p<0.0001$) increased. The cholesterol redistribution was a direct effect of the substantial serum CETP activity levels (38±3 nmol/mL/h) induced by macrophage CETP production.

Conclusions: Macrophage CETP is an important contributor to plasma CETP activity and promotes the development of atherosclerotic lesions.

Funding: This study was supported by the Netherlands Heart Foundation (2001T041).

Tu-W16:3 NORMAL ENDOTHELIAL FUNCTION IN CARRIERS OF THE APOLIPOPROTEIN A-IMILANO MUTANT DESPITE LOW HDL-CHOLESTEROL LEVELS

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Objective: Carriers of apolipoprotein A-IMilano (A-IM), the R173C mutant of apoA-I, are protected against cardiovascular diseases despite very low HDL-cholesterol (HDL-C) levels and hypertriglyceridemia. Aim of the present study was to investigate the endothelial function of A-IM carriers, since low HDL-C levels have been associated with features of endothelial dysfunction.

Methods and Results: Plasma concentrations of soluble cell adhesion molecules (sCAMs), and forearm arterial compliance during reactive hyperemia were evaluated in 21 A-IM carriers, 21 healthy subjects with low HDL-C, and 42 controls. Low HDL-C subjects had significantly higher plasma sCAM levels than controls; on the contrary, no differences were detected between A-IM carriers and controls, indicating that HDL from A-IM carriers may be more efficient than control HDL in inhibiting endothelial CAM expression. Thus, HDL were isolated from 6 A-IM carriers and 6 controls, and their ability to inhibit VCAM-1 expression was tested in endothelial cells. HDL from A-IM carriers were twice effective than control HDL in reducing TNFalpha-induced VCAM-1 expression, and the inhibition occurred at a transcriptional level, as demonstrated by RT-PCR. Interestingly, no differences in arterial compliance during reactive hyperemia were detected between A-IM carriers and controls.

Conclusion: Despite very low HDL-C levels, A-IM carriers do not display features of endothelial dysfunction, such as the increase of circulating sCAM levels and the impairment of arterial compliance, probably because of a superior ability of A-IM containing HDL to protect the endothelium.