Abstracts

Arteriosclerosis, Thrombosis, and Vascular Biology Annual Conference 2007

April 19-21, 2007
Palmer House Hilton
Chicago, IL

Sponsored by the American Heart Association Council on Arteriosclerosis, Thrombosis, and Vascular Biology and the Council on Nutrition, Physical Activity, and Metabolism. Co-sponsored by the National Heart, Lung, and Blood Institute.

Conference Program Committee

Chair: Martha K. Cathcart, PhD, FAHA

William C. Aird, MD; Alan Daugherty, PhD, DSc, FAHA; George E. Davis, MD, PhD; William P. Fay, MD, FAHA; Ronald M. Krauss, MD, FAHA; Mark W. Majesky, PhD; Gwendalyn J. Randolph, PhD; Lawrence L. Rudel, PhD, FAHA; Jonathan Smith, PhD, FAHA; Nancy R. Webb, PhD

Abstracts for the oral and poster presentations are provided in this special on-line supplement.

to oxidized lipids are the mechanisms by which GPx4 inhibits atherogenesis. Unexpectedly, heterozygous mutation to GPx4 did not increase atherosclerotic lesions and oxidized lipids in *ApoE*$^{-/-}$ mice.

P473

Mac-1 Mediates CD40L-induced Atherogenesis

Andreas Zirlik, Christoph Maier, Univ of Freiburg, Freiburg, Germany; Norbert Gerdes, Karolinska Institute, Stockholm, Sweden; Lindsey MacFarlane, Brigham and Women's Hosp, Boston, MA; Juliana Soosairajah, Baker Heart Institute, Melbourne, Australia; Udo Bavendiek, Univ of Hannover, Hannover, Germany; Ingo Ahrens, Baker Heart Institute, Melbourne, Australia; Sandra Ernst, Univ of Freiburg, Freiburg, Germany; Nicole Bassler, Baker Heart Institute, Melbourne, Australia; Anna Missiou, Zsofia Patko, Univ of Freiburg, Freiburg, Germany; Masanori Aikawa, Brigham and Women's Hosp, Boston, MA; Uwe Schönbeck, Boehringer Ingelheim Pharmaceuticals, Ridgefield, CT; Christoph Bode, Univ of Freiburg, Freiburg, Germany; Peter Libby, Brigham and Women's Hosp, Boston, MA; Karlheinz Peter; Baker Heart Institute, Melbourne, Australia

Strong evidence supports a role for CD40L as marker and mediator of inflammatory diseases such as atherosclerosis. Despite extensive characterization of CD40, the classical receptor for CD40L, in immune defense, its role in inflammatory diseases remains uncertain. This study aimed to characterize the contribution of CD40 signaling to atherogenesis. Surprisingly, mice deficient in both CD40 and the low-density lipoprotein-receptor (LDLR) do not develop smaller lesions in the aortic arch, root, and thoraco-abdominal aorta compared to LDLR single-deficient mice that consumed an atherogenic diet for 8 and 16 weeks. Lesions in these two groups of mice also had similar composition. Based on previous reports demonstrating functional binding of GPIIbIIIa to CD40L in thrombosis, we investigated other integrins as potential alternative receptors for CD40L. We demonstrated that CD40L interacts with the integrin Mac-1 on human monocyte/macrophages (using flow cytometry, radioactive binding assays, and immunoprecipitation), resulting in Mac-1-dependent adhesion and migration of inflammatory cells as well as myeloperoxidase release in vitro. Furthermore, mice deficient in CD40L show significantly reduced thioglycolate-elicited accumulation of inflammatory cells in the peritoneal cavity compared with mice deficient in CD40 and wild-type controls. Inhibition of Mac-1 in LDLR-deficient mice attenuates lesion development and reduces lesional macrophage accumulation. These observations identify the interaction of CD40L and Mac-1 as an alternative pathway for CD40L-mediated inflammation. This novel mechanism expands understanding of inflammatory signaling during atherogenesis and has implications regarding novel anti-inflammatory therapies.

P474

Structure-Function of Apolipoprotein A-I$_{Milano}$ and Related Variants

Eric T Alexander, Michael C Phillips, Children's Hosp of Philadelphia, Philadelphia, PA; Daniel J Rader; Univ of Pennsylvania Med Cntr, Philadelphia, PA

Epidemiological studies have shown that plasma apolipoprotein A-I (apoA-I) and high density lipoprotein (HDL) cholesterol levels have an inverse relationship with the risk of developing atherosclerosis. The naturally occurring mutation R173C apoA-I, or apoA-I$_{Milano}$, belies that correlation in that individuals heterozygous for this mutation have reduced plasma levels of apoA-I and HDL, but are not at a greater risk for cardiovascular disease. The hypothesis being investigated here is that the altered cardioprotective ability of apoA-I$_{Milano}$ is specifically related to the introduction of a cysteine residue into the apoA-I primary sequence at position 173 and not the loss of the arginine residue. To address this question, we engineered two mutations of apoA-I, R173K apoA-I and R173S apoA-I. R173K apoA-I is a conservative mutation that maintains the positive charge at position 173 whereas R173S apoA-I closely mimics the apoA-I$_{Milano}$ mutation in that the structure of serine is very similar to cysteine, except for the presence of a hydroxyl group rather than a sulfhydryl group. The proteins have been physically characterized. ApoA-I$_{Milano}$ is less stable than WT apoA-I and has less alpha-helical content. R173S apoA-I resembles apoA-I$_{Milano}$ while R173K apoA-I is almost identical to WT apoA-I. Adeno-associated viruses containing the mutant apoA-I genes were generated and used to infect apoA-I -/- mice. The plasma lipoprotein profile of mice expressing R173K apoA-I was indistinguishable from that of mice expressing WT apoA-I. However, mice expressing either R173S apoA-I or apoA-I$_{Milano}$ had reduced apoA-I levels and HDL cholesterol levels compared to WT apoA-I. Mice expressing WT apoA-I had plasma HDL cholesterol concentrations of 80 $\pm$ 13mg/dl while mice expressing apoA-I$_{Milano}$ had only 31 $\pm$ 2mg/dl. Interestingly, mice expressing R173S apoA-I displayed an intermediate phenotype with plasma HDL cholesterol levels of 48 $\pm$ 5mg/dl. In summary, the altered structure and function of apoA-I$_{Milano}$ is due both to the loss of a positive charge at position 173 and to the introduction of the cysteine residue which allows the formation of disulfide bonds.

P475

A C-Terminal Truncated Human Apolipoprotein A-V Displays Unique Structural and Lipid-Binding Properties

Jennifer A Beckstead, Children's Hosp, Oakland Rsch Institute, Oakland, CA; Kasuen Wong, Univ of California, Berkeley, Berkeley, CA; Vinita Gupta, Children's Hosp, Oakland Rsch Institute, Oakland, CA; Chung-Ping L Wan, California State Univ, Long Beach, Long Beach, CA; Victoria R Cook, Richard B Weinberg, Wake Forest Univ Sch of Medicine, Winston-Salem, NC; Paul M Weers, California State Univ, Long Beach, Long Beach, CA; Robert O Ryan; Children's Hosp, Oakland Rsch Institute, Oakland, CA

Human apolipoprotein A-V (apoA-V) is a potent modulator of plasma triacylglycerol (TG) levels. To probe different regions of this 343 amino acid protein, 4 single Trp apoA-V variants were prepared. The variant with a Trp at position 325, distal to the tetra-proline sequence at residues 293–296, displayed an 8 nm blue shift in wavelength of maximum fluorescence emission upon lipid association. To evaluate the structural and functional role of this 51-residue C-terminal segment, a truncated apoA-V, comprising amino acids 1–292, was generated. Far UV circular dichroism spectra of full-length apoA-V and apoA-V(1–292) were similar with ~50% alpha helix content. In guanidine HCl denaturation experiments, both full-length and truncated apoA-V yielded biphasic profiles consistent with the presence of two structural domains. The denaturation profile of the lower stability component, but not the higher stability component, was affected by the truncation. In fluorescent dye binding experiments, apoA-V(1–292) contained fewer solvent exposed hydrophobic sites than full-length apoA-V. Truncated apoA-V displayed an attenuated ability to solubilize DMPC phospholipid vesicles compared to full-length apoA-V yet it bound to a triolein/water interface with faster kinetics. Taken together, the data support the concept that the 51 amino acid segment C-terminal to the tetra-proline sequence, is not required for apoA-V to adopt a folded protein structure yet functions to modulate apoA-V lipid binding activity and thereby, may be relevant to the mechanism whereby it influences plasma TG levels.

P476

Disruption of Glycosylation at Asparagine-116 of Endothelial Lipase Enhances Activity and Lipoprotein Lipid Hydrolysis in Vitro and in Vivo

Robert J Brown, Gwen C Miller, Nathalie Griffon, Christopher J Long, Daniel J Rader; Univ of Pennsylvania, Philadelphia, PA

We previously identified that four of five putative N-linked glycosylation sites of human endothelial lipase (EL) are utilized, and showed that substitution of the carbohydrate-linked Asn-116 with Ala (N116A) increased the hydrolytic activity of EL. We expanded this observation by first assessing the catalytic activities of additional mutants of the Asn-Asn-Thr glycosylation sequence: Asn-116 to Thr (N116T), Asn-117 to Ala (N117A), and Thr-118 to Ala (T118A). The specific activities of N116T- and T118A-EL were significantly enhanced toward dipalmitoylphosphatidyl choline (250 $\pm$ 30% and 372 $\pm$ 33%, respectively, $p<0.001$) versus both wild-type (WT)- and N117A-EL (100 $\pm$ 25% and 121 $\pm$ 11%, respectively). The specific activities of N116T- and T118A-EL toward triolein were also significantly greater (294 $\pm$ 14% and 269 $\pm$ 6%, respectively, $p<0.001$) versus both WT- and N117A-EL (100 $\pm$ 20% and 100 $\pm$ 26%, respectively). These data demonstrate that it is the loss of glycosylation at Asn-116, and not a structural effect of specifically the N116A mutant, that results in enhanced catalytic activity of EL. We next assessed the hydrolysis of native lipoprotein lipids by N116A-EL. Compared to WT-EL, the N116A mutant exhibited a significant 5-fold increase in low density lipoprotein (LDL) hydrolysis and a 1.8-fold increase in high density lipoprotein (HDL) 2 hydrolysis; HDL_3 hydrolysis was unchanged. Consistent with these observations, adenoviral-mediated expression of N116A-EL (3 x 10^{10} virus particles per mouse) in LDL-receptor-null mice significantly reduced levels of both HDL cholesterol (8.3 $\pm$ 1.9 mg/dl, $p<0.03$) and non-HDL cholesterol (37.8 $\pm$ 9.7 mg/dl, $p<0.03$) beyond the reductions observed by the expression of WT-EL alone (30.8 $\pm$ 9.7 and 81.8 $\pm$ 13.8 mg/dl, respectively). Finally, we introduced Asn-116 of EL into the analogous positions within lipoprotein lipase (LPL) and hepatic lipase (HL), resulting in N-linked glycosylation at this site. Glycosylation at this site significantly suppressed LPL hydrolysis of synthetic substrates, LDL, HDL_2, and HDL_3, but had little effect on HL activity. Overall, these data show that N-linked glycosylation at Asn-116 of EL, or the analogous site of LPL, reduces hydrolysis of lipids within synthetic and lipoprotein substrates.

P477

Normal Endothelial Function in Carriers of the Apolipoprotein A-IMilano Mutant Despite Low HDL-cholesterol Levels

Monica Gomaraschi, Paola Conca, Damiano Baldassarre, Sonia Eligini, Cesare R Sirtori, Guido Franceschini, Laura Calabresi; Univ of Milano, Milano, Italy

Carriers of the apolipoprotein A-IMilano (apoA-IM) mutant show severe reductions in the plasma concentration of antiatherogenic HDL but do not present with preclinical atherosclerosis and premature CHD. Aim of the present study was to investigate endothelial function in A-IM carriers, since low HDL-C levels have been associated with features of endothelial dysfunction. Plasma concentrations of soluble cell adhesion molecules (sCAMs) and forearm arterial compliance (FAC) 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 (sVCAM-1: 656.3$\pm$49.3 vs 502.6$\pm$25.5 ng/ml; sICAM-1: 335.6$\pm$21.5 vs 267.0$\pm$8.9 ng/ml; sE-selectin: 62.9$\pm$4.1 vs 47.9$\pm$3.0 ng/ml); on the contrary, no differences were detected between A-IM carriers (sVCAM-1: 550.6$\pm$32.1 ng/ml; sICAM-1: 309.8$\pm$26.9 ng/ml; sE-selectin: 52.3$\pm$4.3 ng/ml) and controls. Low HDL-C subjects had lower FAC than controls, while no differences were detected between A-IM carriers and controls. These results suggest that HDL from A-IM carriers may be more efficient than control HDL in modulating endothelial function. To test this hypothesis, plasma HDL were isolated from 6 A-IM carriers and 6 controls, and their ability to inhibit VCAM-1 expression and to induce eNOS was tested in cultured endothelial cells. A-IM HDL were two times more effective than control HDL in reducing TNFalpha-induced VCAM-1 expression; the inhibition occurred at a transcriptional level, as demonstrated by RT-PCR. In addition, cells exposed to A-IM HDL showed higher expression of eNOS than cells treated with control HDL. In conclusion, despite the 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 HDL to protect the endothelium.

P478

The Interhelical Sequence Between Helices 7 and 8 of Human Apolipoprotein A-I Influences High-Density Lipoprotein Subclass Association and Generation

Ronald Carnemolla, Andrew Djunaidi, Catherine A Reardon, The Univ of Chicago, Chicago, IL; Jiajun Wang, Wayne State Univ, Detroit, MI; Godfrey S Getz; The Univ of Chicago, Chicago, IL

Humans exhibit a heterogeneous HDL profile (i.e. HDL_2 and HDL_3), in contrast to other species (e.g. mouse) that display a monophasic profile. HDL_2 is thought to be more atheroprotective