865-5 Improvement of Endothelium-Dependent Vasodilation by Simvastatin Is Potentiated by Combination With L-Arginine in Patients With Elevated Asymmetric Dimethylarginine Levels
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Background. Statins stimulate the expression of endothelial NO synthase (eNOS) in vitro and enhance endothelium-dependent, NO-mediated vasodilation in vivo. Asymmetric dimethylarginine (ADMA) is an endogenous, competitive inhibitor of eNOS. The presence of elevated plasma ADMA levels is associated with endothelial dysfunction. We investigated the hypothesis that simvastatin may enhance endothelial function in patients with elevated ADMA only if the inhibitory effect of ADMA is overcome by supplemental L-arginine.

Methods. 15 clinically asymptomatic, elderly subjects with elevated ADMA levels received, in a randomised order, simvastatin (40 mg/day), L-arginine sustained-release (3 g/day), or a combination of both, each for 3 weeks, in a three period crossover design with at least three weeks of wash-out between treatments. Endothelium-dependent vasodilation was assessed by brachial artery ultrasound using computer-assisted image analysis; ADMA and L-arginine plasma concentrations were determined by a validated HPLC method.

Results. Analysis of 12 subjects who completed the study revealed that simvastatin had no effect on endothelium-dependent vasodilation when administered alone (6.2±1.2% vs. 6.1±0.9%) but significantly improved endothelial function (8.7±0.7 vs. 4.9±0.8%; p<0.02). When given in combination with L-arginine, simvastatin had a significant beneficial effect on endothelial function (8.9±1.5 vs. 5.3±0.8%; p<0.01). Endothelium-dependent vasodilation by glycyl trinitrate was not affected by any of the treatments. L-arginine, either alone or in combination with simvastatin, significantly improved plasma L-arginine/ADMA ratio (baseline, 82.3±4.0 vs. 102.8±9.2 and 102.6±10.8, respectively, each p<0.05).

Conclusions. Simvastatin does not enhance endothelial function in subjects in whom eNOS is blocked by elevated ADMA levels; combination of simvastatin with oral L-arginine has a synergistic effect on endothelial function. ADMA-mediated effects may play a major role in therapeutic effects of statins, combination with L-arginine should be considered in patients with elevated ADMA concentration.

865-4 Tetrahydrobiopterin Prevents Vascular Injury After Ischemia-Reperfusion in Humans
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BACKGROUND: Vascular inflammation and subsequent endothelial dysfunction are pivotal steps in the initiation of ischemia-reperfusion injury. Ischemic preconditioning is well known to have protective effects although the mechanism through which it occurs remains incompletely understood. We hypothesized that tetrahydrobiopterin (BH4) plays a critical role in ischemic preconditioning, and its administration prevents reperfusion injury.

METHODS: Baseline endothelial function of the radial artery was measured in 21 healthy volunteers (mean age 36.2±9.6 years) using flow-mediated dilation (FMD). Subjects were divided into 3 groups: A) arm ischemia induced by a 15 minute cuff inflation B) ischemia-reperfusion with preconditioning with 3 5-minute cuff inflations followed by 20 minute cuff inflation and C) administration of BH4 (500 µg/min) into the brachial artery followed by 20 minute cuff inflation. FMD was measured in all groups after 15 minutes of reperfusion.

RESULTS: Twenty minutes of cuff inflation followed by reperfusion induced ischemic vascular injury as evidenced by impaired FMD (pre 7.4±0.6%, post -0.5±0.9%, p<0.001). In all, ischemic preconditioning preserved endothelial function (FMD pre 6.8±0.6%, FMD post 7.4±1.0%, p=0.6, NS). Importantly, administration of BH4 also reduced the development of endothelial dysfunction (FMD post 7.3±0.8%, FMD post 4.8±0.7%, p=0.03; p=0.02 compared with post-FMD of the ischemia-reperfusion group).

CONCLUSIONS: Ischemia-reperfusion produces significant endothelial dysfunction in humans that can be prevented by ischemic preconditioning. Reperfusion injury is also dramatically attenuated by the administration of BH4. These findings suggest that reperfusion vascular injury occurs secondary to BH4 depletion and the uncoupling of eNOS.

865-3 Determinants of Endothelial Function and Arterial Stiffness
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Background. Experimental data suggest that the endothelium-mediated, flow-dependent vasodilatation of peripheral arteries (FDD) is not only mediated by nitric oxide (NO) but also by other relaxing factors, such as the cytochrome-p450-dependent endothelium-derived hyperpolarization factor (EDHF). However, the contribution of EDHF for the endothelium-mediated vasodilatation of peripheral arteries in humans is unknown.

Methods. FDD of the radial artery was determined in 12 healthy volunteers by high resolution ultrasound. To inhibit vascular cyclo-oxygenase dependent vasodilatation, we injected 500 mg acetylsalicylic acid 30 min before FDD-measurements. FDD was analyzed during control conditions and after intra-arterial infusion (5 min) of sulfaphenazole, a specific inhibitor of the isoenzyme 2C9 of the cytochrome-p450 (4 mg/min), following L-NMMA (NO-synthase-inhibitor, 7 µmol/min) and after co-infusion of both, each 5 min. Furthermore, endothelium-independent vasodilatation was characterized after intra-arterial infusion of SNP (8 µg/min, 5 min).

Results. FDD at baseline was 11.5±3%, following sulfaphenazole 7.4±3.3% (p<0.01 vs. control), after L-NMMA 6.0±2.4% (p<0.01 vs. control), after co-infusion of L-NMMA and sulfaphenazole 3.9±2.5% (p<0.01 vs. control, p<0.05 vs. L-NMMA); p<0.01 vs. sulfaphenazol). Sulfaphenazole had no effect on endothelium-independent vasodilatation (SNP: 19.6±6.2%, SNP+sulfaphenazole:20.2±6.8, p=n.s.).

Conclusion. FDD of the radial artery was substantially reduced in healthy volunteers after inhibition of cytochrome-p450 2C9 or NO-synthase. Co-infusions of both inhibitors for NO-synthase and EDHF had incremental inhibitory effects on FDD. Thus, our results support the concept that EDHF contributes to flow-dependent dilatation of peripheral conduit arteries in normal human volunteers in vivo.

8:45 a.m.

865-2 Influence of Statin on Endothelial Function Is Modulated by 1166A/C Polymorphism of the Angiotensin AT1 Receptor Gene in Healthy Volunteers
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Background. Angiotensin AT1 receptors (AT1R) may influence function of vascular endothelium via stimulation of free radicals production. We examined relationship between 1166C/A polymorphism of the AT1R gene and statin related changes in endothelial function and AT1R density.

Methods. In 17 pts with coronary artery disease (not on hypolipemic treatment) lipid profile, platelet AT1R density and endothelial function (brachial artery flow mediated dilation FMD), NO metabolites: nitrate&nitrite concentration) were performed at baseline and after treatment with simvastatin 40mg/24h for 12 weeks. All subjects were genotyped for the 1166C/A polymorphism.

Results. At baseline there were no differences between pts carrying AA allele (n=9) vs. C allele (AC and CC; n=8) in LDL cholesterol, AT1R density and FMD. Pts carrying AA allele had at baseline lower nitrate&nitrite concentration (13.8±4.4 vs. 21.39±9.45µM; p=0.04). After simvastatin AA pts had significant improvement in endothelial function while AC or CC pts had no improvement in endothelial function (table). There were no differences between AA homoygotes and all C allele carriers in simvastatin-induced reduction in LDL cholesterol and AT1R density.

Conclusion. Influence of statin on endothelial function is modulated by 1166C/A polymorphism of the angiotensin AT1R gene. This polymorphism does not affect (i) the baseline AT1R density and baseline LDL, (ii) simvastatin induced reduction of LDL and AT1R density.

8:45 a.m.
Nitric Oxide Synthase Gene Polymorphism (G894T) Peroxynitrite Inactivates Akt Pathway and Enhances Background:

Center, New Orleans, LA, University of Texas-Houston Health Science Center, Houston, Shengxu Li, Eric Boerwinkle, Gerald S. Berenson, Tulane University Health Sciences Spleen cells from atherosclerotic mice exhibited lowered constitutive expression of ICOS ICOS was abundantly expressed within plaques of humans and ApoE-KO mice and colo-
tors and its expression in splenocytes. ApoE-KO mice were immunized with human
mice and treated with simvastatin (25µM) or gera-
nylgeranyl transferase inhibitor (GGTI-298 5µM). We found, using fractionation by ultra-
centrifugation and immunofluorescence, both inhibitors of prenylation altered subcellular localization, shifting RacV12 from 96% membrane localized to 2% and 4.1% with simvas-
tatin and GGTI respectively. Prenylation inhibition had minimal effect on “Rac activity”
determined by PAK-PBD affinity precipitation of total lysate and supersede production
but instead strongly shifted activity from the membrane to the cytoplasmic fraction. Untreated RacV12 activated Akt, but simvastatin and GGTI treatment further increased
p-AKT by two fold. Physiologically, this activation was mimicked by a two-fold inhibition of
serum starvation induced apoptosis in RacV12 cells treated with simvastatin or GGTI
compared to RacV12 alone (RacV12 25% decrease relative to CT, RacV12+simvastatin
82%, RacV12+GGTI 55%). In contrast, simvastatin and GGTI had no effect on apoptosis
in quiescent adenoiral null control cells where there is minimal Rac activity. Conclu-
sions: These data support a novel understanding of Rac signaling, subcellular localiza-
tion, and its modulation by statins that may better explain the pleotropic effects of statins.
Rather than globally inhibiting Rac, prenylation deficient Rac appears to become cytosol-
ically distributed where it remains “active” and has a more potent ability to stimulate
endothelial cell survival through Akt phosphorylation that may enhance plaque stability
and protect from atherosclerosis.

Background: The endothelial nitric oxide synthase (eNOS) gene is known to influence the regulation of blood pressure levels. However, whether the eNOS gene locus influ-
ences arterial stiffness, independently of blood pressure, is unknown.

Methods: Arterial stiffness was measured in 118 black and 285 white young adults aged 25-37 years from M-mode ultrasounds of common carotid artery using Petersen’s (Ep) and Young’s (YEM) elastic modulus.

Results: Blacks displayed a lower frequency of the T allele than whites (0.131 vs 0.321, P=0.001). The T allele was associated with lower systolic blood pressure in blacks (P=0.04), but not in whites. Blacks showed significantly higher values of Ep (i.e. increased stiffness) than whites (49.9 kPa vs 45.5 kPa, P=0.003); whereas no such race difference was found for YEM, a measure of elastic adjustment for relative wall thickness. After controlling for sex, age, BMI and mean arterial pressure, the T allele was associ-
ated with significantly lower values of Ep (P=0.012) and YEM (P=0.034) in blacks. Although similar trends were seen in whites, the genotype effect on Ep and YEM was not significant. In the total sample, including race as an additional covariate, the G894T gen-
otype was associated with Ep (P=0.051) and YEM (P=0.038).

Conclusion: These results suggest that the G894T polymorphism at the eNOS gene locus is associated with lower arterial stiffness, adjusting for blood pressure levels, in asymptomatic young adults, especially in blacks.

Vascular Biology: Cell Signalling, Atherosclerosis, and Thromboembolism Wednesday, March 10, 2004, 8:30 a.m.-10:00 a.m. Morial Convention Center, Room 243

A Functional Role for Inducible Costimulator in Atherosclerosis

Jacob George, Gad Keren, Tel Aviv Medical Center, Tel Aviv, Israel

Background: Lymphocytes appear to influence atherosclerosis by altering cytokine pro-
duction. Whereas primary lymphocyte activation requires T cell receptor ligation, costim-
ulatory signals also appear requisite for generation of a functional T cell response. Inducible costimulator (ICOS) is a newly discovered T cell molecule with a dual role in immune mediated disorders. Herein, we tested the importance of ICOS in atherosclero-
sion, and its expression in splenocytes. ApoE-KO mice were immunized with human ICOS/Fc-chimera or non-fused Fc and either provided a chow diet for 6 weeks, or a high
diet for 8 weeks.

ICOS was abundantly expressed within plaques of humans and ApoE-KO mice and colo-
ized with CD3 cells whereas ICOS ligand was expressed in plaque macrophages. Spleen cells from atherosclerotic mice exhibited lowered constitutive expression of ICOS yet priming with oxLDL enhanced ICOS expression dose-dependently.

In mice induced to develop fatty streaks and to generate ICOS blocking antibodies, early atherosclerosis was increased by ~77% whereas upon inducing more advanced lesions, the increase in plaque area upon ICOS blockade group was ~36%. IFN-γamma secretion by oxLDL-primed splenocytes in ICOS-immunized mice increased whereas IL-10 secre-
tion diminished as compared to control animals. A similar trend in cytokine production was evident in the lesion by immunohistochemistry.

Conclusion: ICOS appears as an influential costimulatory pathway in atherosclerosis that may play a protective rather that a proatherogenic role.