ORAL CONTRIBUTIONS

865

Determinants of Endothelial Function and Arterial Stiffness

Wednesday, March 10, 2004, 8:30 a.m.-10:00 a.m.
Morial Convention Center, Hall E-1

8:30 a.m.

865-1 Endothelium-Derived Hyperpolarizing Factor Is Involved in Flow-Dependent Dilation of Peripheral Conduit Arteries in Healthy Volunteers

Dieter Fischer, Stephan Spiekermann, Ulf Landmesser, Marian Hospely, Smita Jategaonkar, Ingrid Fleming, Helmut Drexler, Burkhard Hornig, Medizinische Hochschule Hannover, Hannover, Germany, J.W. Goethe-Universität, Frankfurt, Germany

Background: Experimental data suggest that the endothelium-mediated, flow-dependent vasodilation 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 vasodilation 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 cyclooxygenase dependent vasodilation, we injected 500 mg acetylsalecyl 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 vasodilation was characterized after intra-arterial infusion of SNP (9 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% (<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. sulfaphenazole). Sulfaphenazole had no effect on endothelium-independent vasodilation (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 dilation of peripheral conduit arteries in normal human volunteers *in vivo*.

8:45 a.m.

865-2 1166 A/C Polymorphism of the Angiotensin AT1 Receptor Gene Alters Simvastatin-Induced Change in the Endothelial Function

Marek Kiliszek, Michal Maczewski, Beata Burzynska, Grzegorz Styczynski, Monika Duda, Andrzej Beresewicz, Grzegorz Opolski, Medical University of Warsaw, Warsaw, Poland, Medical Centre of Postgraduate Education, Warsaw, Poland

Background: Angiotensin AT1 receptors (AT1R) may influence function of vascular endothelium via stimulation of free radicals production. We examined relationship between 1166A/C 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 1166A/C 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 (13,84±4,43 vs. 21,39±9,45uM; p=0,048). 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 homozygotes and all C allele carriers in simvastatin-induced reduction in LDL cholesterol and AT1R density.
Conclusions: Influence of statin on endothelial function is modulated by 1166A/C 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.
Table:

Index of endothelial function	Patients carrying AA allele			Patients carrying AC or CC allele		
	Before simvastatin Mean(SD)	After simvastatin Mean(SD)	p	Before simvastatin Mean(SD)	After simvastatin Mean(SD)	p
FMD (%)	8,27(2,30)	11,25(2,79)	0,012	10,15(7,86)	9,26(4,68)	0,65
nitrate&nitrite concentration	13,84(4,43)	22,21(8,53)	0,01	21,39(9,45)	20,00(9,13)	0,44

9:00 a.m.

865-3 Improvement of Endothelium-Dependent Vasodilation by Simvastatin Is Potentiated by Combination With L-Arginine in Patients With Elevated Asymmetric Dimethylarginine Levels

Gerhild I. Boger, Renke Maas, Edzard Schwedhelm, Anneke Bierend, Ralf Benndorf, Mariola Kastner, Anna Steenpaß, Rainer H. Boger, University Hospital Hamburg-Eppendorf, Hamburg, Germany

Background. Statins stimulate the expression of endothelial NO synthase (eNOS) in vitro and enhance endothelium-dependent, NO-mediated vasodilation in vivo. Asymmetrical 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%). L-arginine 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 (9.8±1.5 vs. 5.3±0.8%; p<0.01). Endothelium-independent vasodilation by glyceryl 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. As NO-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.

9:15 a.m.

865-4 Tetrahydrobiopterin Prevents Vascular Injury After Ischemia-Reperfusion in Humans

Vasilis C. Babaliaros, Arshed A. Quyyumi, Uma P. Reddy, W. Lance Lewis, Sonya L. Lefever, W. Robert Taylor, Emory University School of Medicine, Atlanta, GA

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±2.9 years) using flow-mediated dilation (FMD). Subjects were divided into 3 groups: A) arm ischemia induced by a 20 minute cuff inflation B) preconditioning with three 5-minute cuff inflations followed by 20 minute cuff inflation and C) administration of BH4 (500µg/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 addition, 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 pre 7.32±0.8%, FMD post 4.8±0.7%, p=0.03; p=0.002 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.

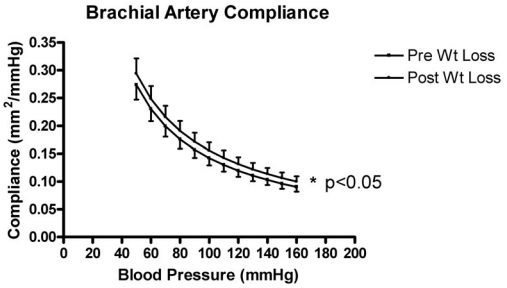
9:30 a.m.

865-5 Six Months of Weight Loss Improves Metabolic and Vascular Indices in Overweight Adults

Thomas P. Olson, Aaron S. Kelly, Alan J. Bank, Daniel R. Kaiser, Donald R. Dengel, University of Minnesota, Minneapolis, MN, St. Paul Heart Clinic, St. Paul, MN

Background: Obesity is a risk factor for cardiovascular disease and is characterized by metabolic and vascular abnormalities.We examined the effects of weight loss on metabolic and cardiovascular parameters. **Methods:** Twelve (F=9, M=3) overweight (BMI 30.3±3.7) adults (54.9±3.9 yr) without diabetes or vascular disease were counseled by a registered dietician to lose weight over six months. Vascular structure, function, and wall mechanical properties were measured via ultrasound. Insulin sensitivity (IVGTT), body composition (dual-energy x-ray absorptiometry), and lipids were also assessed. **Results:** There were significant reductions in body mass (86.3±14.2 vs. 79.5±13.8 kg, p<0.0001) and percent fat (44.3±7.0 vs. 41.0±8.5%, p<0.01) after weight loss. There were significant improvements in total cholesterol (230.9±33.9 vs. 194.8±30.7 mg/dl, p<0.0001), LDL-C (149.3±27.2 vs. 123.7±24.0 mg/dl, p<0.0001), triglycerides (131±88.6 vs.

93.5±33.6 mg/dl, p<0.05), and insulin sensitivity (3.3±1.7 vs. 5.4±1.6 μU x 10^{-4} · min^{-1} · ml^{-1}, p<0.0001) following weight loss. Brachial artery compliance (p<0.05) and distensibility (p<0.05) curves over the physiological pressure range improved (figure), whereas endothelial function and intima-media thickness remained unchanged. **Conclusions:** In overweight adults, six months of weight loss resulted in improvements in body composition, insulin sensitivity, lipid profile, and brachial artery compliance and distensibility.



9:45 a.m.

865-6 **Nitric Oxide Synthase Gene Polymorphism (G894T) Influences Arterial Stiffness in Adults: The Bogalusa Heart Study**

Wei Chen, Sathanur R. Srinivasan, M. Gene Bond, Rong Tang, Elaine M. Urbina, Shengxu Li, Eric Boerwinkle, Gerald S. Berenson, Tulane University Health Sciences Center, New Orleans, LA, University of Texas-Houston Health Science Center, Houston, TX

Background: The endothelial nitric oxide synthase (eNOS) gene is known to influence the regulation of blood pressure levels. However, whether the eNOS gene locus influences 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 Peterson'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 elasticity adjusted for relative wall thickness. After controlling for sex, age, BMI and mean arterial pressure, the T allele was associated 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 genotype 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 wall stiffness, adjusting for blood pressure levels, in asymptomatic young adults, especially in blacks.

ORAL CONTRIBUTIONS

867

Vascular Biology: Cell Signalling, Atherosclerosis, and Thromboembolism

Wednesday, March 10, 2004, 8:30 a.m.-10:00 a.m.
Morial Convention Center, Room 243

8:30 a.m.

867-1 **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 production. Whereas primary lymphocyte activation requires T cell receptor ligation, costimulatory 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 atherosclerosis.
Methods and results: Atherosclerotic plaques from humans and ApoE-KO mice were studied immunohistochemically for the presence and localization of ICOS and its receptors 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 fat diet for 8 weeks.
ICOS was abundantly expressed within plaques of humans and ApoE-KO mice and colocalized 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-gamma secretion by oxLDL-primed splenocytes in ICOS-immunized mice increased whereas IL-10 secretion 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.

8:45 a.m.

867-2 **Simvastatin Augments Rac Activation of Akt Signaling and Inhibits Endothelial Apoptosis by Modifying Subcellular Localization**

David Gregg, Neuza H. Lopes, Pascal J. Goldschmidt, Duke University Medical Center, Durham, NC

Background: HMG Co-A Reductase inhibitors (statins) inhibit atherosclerosis to a greater degree than would be predicted by lipid reduction alone.One focus has been on the ability of statins to block prenylation of the Rho GTPase Rac to inhibit its signaling. We **hypothesized** that rather than completely inhibiting Rac signaling, statins selectively modulate Rac by altering subcellular localization. **Methods and Results**: To test the hypothesis that unprenylated cytosolic Rac may activate signaling pathways unique from cellular membrane localized Rac, we overexpressed a constitutively active RacV12 mutant in human aortic endothelial cells and treated with simvastatin (25μM) or geranylgeranyl transferase inhibitor (GGTI-298 5μM). We found, using fractionation by ultracentrifugation and immunofluorescence, both inhibitors of prenylation altered subcellular localization, shifting RacV12 from 59% membrane localized to 2% and 4.1% with simvastatin and GGTI respectively. Prenylation inhibition had minimal effect on "Rac activity" determined by PAK-PBD affinity precipitation of total lysate and superoxide production but instead strongly shifted activity from the membrane to the cytoplasmic fraction. Untreated Rac V12 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 Rac V12 cells treated with simvastatin or GGTI compared to RacV12 alone (Rac V12 25% decrease relative to CT, RacV12+simvastatin 62%, RacV12+GGTI 55%). In contrast, simvastatin and GGTI had no effect on apoptosis in quiescent adenoviral null control cells where there is minimal Rac activity. **Conclusions**: These data support a novel understanding of Rac signaling, subcellular localization, 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 cytosolically 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.

9:00 a.m.

867-3 **Peroxynitrite Inactivates Akt Pathway and Enhances Tissue Factor Expression in Thrombin Stimulated Endothelial Cells**

Maria Berrozpe, Masato Eto, Hana Joch, Csaba Szabó, Thomas Felix Lüscher, Cardiovascular Research, Institute of Physiology, University of Zurich, Zurich, Switzerland, Inotek Pharmaceuticals Corporation, Beverly, MA

Background: Tissue Factor (TF) plays a pivotal role in thrombus formation in acute coronary syndromes. Peroxynitrite (NOO^-) modifies protein activity by nitration of certain amino acids. However the influence of peroxynitrite in TF expression and activity and the signaling pathways involved in this phenomena remains unclear. **Aim:** To elucidate the effect of NOO^- on TF and the role that Akt has in this effect. **Methods and Results**: Internal mammary artery endothelial cells were incubated with SIN-1 (NOO^- donor), FP15 (NOO^- catalyst), TEMPOL (superoxide scavenger)and wortmmanin (PI3K inhibitor) during 1 hour. Afterwards, thrombin was added. Ten minutes after thrombin stimulation Akt phosphorylation (measured by western blotting) decreased and 5 hours later, TF expression (measured by western blotting) and activity (Measured by American Diagnostica kit) increased (Figure A). Wortmannin and SIN-1 potenciated this effect. Not only potenciation of SIN-1 but also thrombin effect was reversed by coincubation with FP15 and TEMPOL. NOO^- staining by DCF demonstrated a significant increase in thrombin stimulated cells (Figure B, DCF:green, endothelial cell marker CD31: red, nuclei: blue). **Conclusions:** These data demonstrate an inhibitory effect of NOO^- in Akt phosphorilation that potentiates thrombin induced TF expression and activity in human endothelial cells. Thus, oxidative stress with formation of NOO^- from nitric oxide may be thrombogenic via this pathway.