POSTER SESSION

1140

Biologic Mechanisms of Atherosclerosis

Tuesday, March 09, 2004, Noon-2:00 p.m.
Morial Convention Center, Hall G
Presentation Hour: 1:00 p.m.-2:00 p.m.

1140-165 Long-Term Statin Treatment Protects Against Nitrate-Induced Oxidative Stress

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Background:Nitrate tolerance is associated with an enhanced superoxide anion production and could be attenuated by statins as they interact with the two main (eNOS and NAD(P)H oxidase) pathways involved in this oxidative stress.
Methods: 3 groups of normocholesterolemic rats were treated; group 1 received rosuvastatin (10 mg/kg/d p.o) for 5 weeks and the last 3 days a cotreatment with the statin plus nitroglycerin (NTG 50 mg/kg/d, sub-cutaneous injections b.i.d.); group 2 received only NTG (50 mg/kg/d, b.i.d. for 3 days) and group 3 served as control. Rings of thoracic aortas from these groups were studied in organ baths. Relaxations to NTG (0.1 nM to 0.1 mM) were determined on phenylephrine-preconstricted rings and O2- production (counts/10s/mg) was assessed by lucigenin chemiluminescence technique.
Results: In group 2 (NTG), the concentration-response curves to NTG were significantly shifted to the right : the pD2 (-log NTG concentration evoking a half maximal relaxation) was 6.76+/-0.06 (n=7) vs 7.77+/-0.08 (n=7) in group 3 (not exposed to NTG, P<0.01) ; O2- production was enhanced (289+/-23 (n=6) vs 183+/-34 (n=5, P<.05)). In contrast, in group 1, the rightward shift was attenuated (pD2 values was 7.19+/-0.11 (n=8), P<.05 vs group 2); O2- production was decreased : 208+/-19 (n=6, P<.05 vs group 2). In addition, before NTG exposure, rosuvastatin treatment decreased p22phox (the essential NAD(P)H subunit) abundance in aortic wall and NAD(P)H oxidase activity. In contrast, this treatment did not alter either eNOS abundance or the basal release of endothelium-derived NO.
Conclusion: Long-term rosuvastatin treatment protects against nitrate tolerance by counteracting NTG-induced increase in O2- production. This protection seems to involve a direct interaction with the NAD(P)H oxidase pathway rather than an upregulation of the eNOS pathway.

1140-166 Enhancing Arteriogenesis Increases Atherosclerosis and Reducing Atherosclerosis Reduces Arteriogenesis: Demonstration of the Concept in Mice

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Background: Inflammatory and immune cells are known to contribute to atherogenesis. We and others have reported data indicating that such cells also enhance collateral development. We have used two mouse models with different predispositions to both atherosclerosis and collateral development to gain insight into this "trade-off" phenomenon. It is our hypothesis that: *Shared inflammatory mechanisms contribute to atherogenesis and to arteriogenesis; stimulating these pathways will activate both processes.* Consideration of these findings will be important in developing clinical strategies designed to enhance collaterals or prevent atherosclerosis in patients.
Methods: Aortic lesion area was assessed in 16 week, chow fed Balb/c and C57BL/6J ApoE-/- mice (n=20) using Oil Red O staining and computer assisted morphometric analysis. Total serum cholesterol was quantified. To examine collateral development, at 12 weeks, C57BL/6J and Balb/c animals (n=10) underwent left femoral artery ligation. Flow recovery was assessed using Laser Doppler Perfusion Imaging over a 4 week period and expressed as a ratio of ischemic to normal limb flows.
Results: The C57BL/6J ApoE -/- mice had greater lesion area when compared to Balb/c ApoE-/- mice, although the Balb/c ApoE-/- mice had higher serum cholesterol levels. [C57BL/6J ApoE -/- vs. Balb/c ApoE-/-: Lesion area ($\mu m^{2)}$; 22,081 +/-2646 vs. 8062 +/-1429, p< 0.0001, cholesterol levels (mg/dl); 840+/-49 vs. 960 +/-38, p<0.039]. Blood flow recovery was significantly better in C57BL/6J compared to Balb/C [0.652 vs 0.306 at day-7, 0.806 vs 0.519 at day-28, p<0.001 for trend by ANOVA].
Conclusions: The C57BL/6J mouse is more susceptible to atherosclerosis than the Balb/c strain; however, the C57BL/6J strain exhibits a marked improvement in collateral flow during recovery from femoral artery ligation that is not observed in the Balb/c mouse. These data support our hypothesis that shared mechanisms contribute to both atherosclerosis and arteriogenesis, and set the stage for future studies to examine this "trade-off" phenomenon in greater detail.

1140-167 Cytomegalovirus Impairs Endothelial Nitric Oxide Synthase Pathway: Role of Oxidative Stress and Viral Entry

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Background: Seroepidemiological and histopathological evidence has implicated CMV in pathogenesis of atherosclerosis. We hypothesized CMV may accelerate atherogenesis by impairing endothelial nitric oxide synthase (NOS) pathway. An important determinant of NOS activity is ADMA (asymmetric dimethylarginine), an endogenous inhibitor of NOS. Methods and Results: To determine whether CMV impairs the NOS pathway by increasing ADMA accumulation, cultured human microvascular endothelial cells (EC) were infected with endotheliotrophic human CMV strain, TB40/E, at MOI 1, 3, and 10. HPLC analysis of the conditioned medium showed a dose-dependent increase in ADMA accumulation. The increase in ADMA was associated with a decline in intracellular cGMP (by 43%) indicating inhibition of the NOS pathway. We hypothesized accumulation of ADMA was due to its impaired degradation by enzyme dimethylarginine dimethylaminohydrolase (DDAH). Indeed, the CMV-induced increase in ADMA after 72h infection was temporally associated with a marked reduction (over 80%) in DDAH activity, which is impaired by oxidative stress. CMV dose- and time-dependently increased generation of intracellular O_2-derived free radicals using FACS analysis and a fluorescent marker, DCFDA. Surprisingly, the CMV-induced increase of oxidative stress did not require viral replication because UV-inactivated virus induced the same response, but did require binding and entry of viral particles because neutralizing antibody to the virus blocked its effects to increase ADMA. Tegument proteins carried by CMV are known to modulate immunological response in infected cells, and could be responsible for the observed effects. One of these proteins, phosphoprotein 65, was detected within EC by immunofluorescent staining even after exposure to UV inactivated virus. Conclusion: CMV infection impairs the NOS pathway by inducing an oxidative impairment of DDAH which leads to accumulation of the NOS inhibitor ADMA. This effect of CMV on EC does not require viral gene replication, but does require viral binding and entry, suggesting release of one or more viral tegument proteins after viral entry may be responsible for derangement of the NOS pathway.

1140-168 In Vivo Caspase-Inhibition Prevents Plaque Progression by Reducing Apoptosis and Tissue-Factor Expression in a Mouse Model of Chronic Atherosclerosis

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Background: Lipid lowering therapy has been shown to reduce thrombogenicity of atherosclerotic plaques. Inflammatory cells undergoing apoptosis when exposed to ox-LDL in lipid-rich plaque can contribute to plaque thrombogenicity via synthesis of tissue-factor (TF). Therefore, increased intimal apoptosis and tissue-factor expression could be a key factor for accelerating plaque progression. To test this hypothesis we evaluated the effect of caspase-inhibition (CI) in a mouse model of atherosclerosis in vivo.
Methods: Apo-E -/- mice (n=13) were fed a Western Diet for 7 months. Three animals were sacrificed at 7 month and served as baseline control (BL). The remaining animals were randomized into treatment (TR) (n=5) and control group (CT) (n=5) receiving either 3 weeks of continuous CI (daily i.p. injection of 30 mcg/kg of Q-VD-OPH general caspase-inhibitor in 50 mcl DMSO) (n=5) or DMSO treatment alone (n=5) (data as mean±SEM). Plasma cholesterol levels were similar in all groups with 780±58mg/dl for the BSL group, and 958±64mg/dl and 854±56mg/dl for the TR and CT group. Atherosclerosis was assessed by (immuno)histo-morphometric analysis of the aortic root and by Sudan IV stainings of whole-mounted abdominal aorta.
Results: CI significantly reduced plaque size compared to controls (0.59±0.05 vs. 0.88±0.04, P<.01). Lipid deposition (Sudan IV) of abdominal aorta was also significantly reduced by CI compared to CT and BSL. Expression of both aCS-3 and TF in intimal cells was significantly reduced by CI compared to CT and BSL (aCS3: 9%±2 vs. 30%±3, P<.01; 9%±2 vs. 23%±3, P<.01; TF: 15%±2 vs. 41%±3, P<.01, 15%±2 vs. 43%±5, P<.01) and correlated positively with plaque size (P<.05). Double-labeling demonstrated co-localization of TF and aCS-3.
Conclusion: CI inhibited plaque progression and reversed the increased intimal expression of both apoptosis and TF despite persistently elevated blood lipid levels. These findings point to intimal apoptosis and associated tissue-factor expression as a possible final common pathway involved in plaque progression. Therefore, CI could be a powerful tool to protect intimal cells from diverse pro-apoptotic stimuli leading to a better treatment of atherosclerosis.

1140-169 Heavy Coronary Plaque Calcification Is Associated With Only Minor Degrees of Plaque Macrophage and Neovessel Density Than Plaques With Less Calcification: Solving the Calcium Paradox?

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Background. Carotid symptomatic plaques are usually severely calcified. There is greater variability in that respect with the coronary arteries especially in cases of thin cap fibroatheromas leading to cap ruptures, where severe calcification may occur but is not the norm. In the present work we compared plaques with heavy calcification with plaques with less calcification with regards to macrophage infiltration and neovascularization.
Methods. Coronary artery section from ten patients with plaque ruptures and of ten patients with fibrocalcific plaques were obtained from the Armed Forces Institute of Pathology files. Plaques were routinely decalcified prior to processing. We performed immunohistochemistry for detection of neovascularization with the endothelial cell markers factor VIII and for macrophages with the Kp-1 marker. Morphometric computerized analysis was performed with Olympus MicroSuite software
Results. When the calcification was mild, not reaching the superficial third of the plaque, it coexisted with a lipid necrotic core and was accompanied by a significant macrophage infiltration in the plaque and neovascularization (the latter more prominent in the underlying media and adventitia). Areas of heavy calcification (even in the same cross-section) were associated with loss of lipid core, decrease to almost absence of macrophages and neovessels density.
Conclusion. The study suggests a plausible explanation for the apparent paradox of calcium being a marker of plaque vulnerability and at the same time providing protection to the plaque. Mild/moderate calcification is associated with known markers of vulnerable