Simvastatin Preserves the Structure of Coronary Adventitial Vasa Vasorum inExperimental Hypercholesterolemia Independent of Lipid Lowering
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Background: Experimental hypercholesterolemia (HC) is associated with vasa vasorum (VV) neovascularization prior to lesion formation. Studies have been repeatedly reported to have beneficial vascular effects, independent of lipid lowering. Their effect on VV neovascularization, however, is completely unknown.

Methods: Female pigs (28-36 kg) were randomized to either normal diet (N, n=5) or high cholesterol diet without (HC, n=3) or with simvastatin supplementation (40-60 mg per day) (HC+S, n=4) for 12 weeks. The proximal LAD segment was scanned by 3D-MRT and VV density was determined in spiral cross-sections. Coronary artery content of vascular endothelial growth factor (VEGF) was assessed by immunoblotting and -staining.

Results: Compared to N (84±2 mg/dl), plasma cholesterol was increased in HC and HC+S (81±12 and 56±161 mg/dl, p<0.05). Vessel wall area (5.1±0.2 vs. 1.6±0.1 mm²) and VV density (4.7±0.3 vs. 2.7±0.2 mm²) were increased in HC compared to N (p<0.05). This increase in VV density was preserved in HC+S (3.0±0.2 vs. 2.0±0.2 mm², p<0.05). In parallel, increase in tissue expression of VEGF in HC was preserved in HC+S.

Conclusions: Simvastatin attenuates the increase in coronary artery VEGF expression and prevents VV neovascularization in HC, despite no change in plasma lipids. These findings underscore the beneficial effect of statins upon vascular alterations in atherosclerosis, independent of lipid lowering.

1176-73
Accelerated Neointima Formation in a Mouse Injury Model of Type 2 Diabetes Mellitus: Hyperglycemia, Hyperinsulinemia, and Insulin Resistance Following Lipid Feeding in the Apolipoprotein-E-Deficient Mouse

Hyperglycemia(HG) and hyperinsulinemia(HI) enhance the atherosclerotic complications of diabetes. Using a mouse injury model, we hypothesized that apolipoprotein E deficient(C57L/J) mice fed a Western diet(WD) would develop more neointimal thickening compared to mice without MEG treatment.

Methods: Female ApoE-/- mice(n=10/group) were fed WD, FD, or CD for 1 week before injury of the left common carotid artery and continued for 4 weeks. At sacrifice, fasting glucose, insulin and lipids were measured. Pancreatic islets for insulin release profile and aortic sinus for collagen measurement were harvested. Results: Baseline glucose was normal in all groups. At sacrifice, glucose and insulin were higher in the WD group. Insulin release profiles demonstrated loss of the 1st peak and an attenuated 2nd peak in WD group and blunted 1st and 2nd peaks in FD group. Gradation in cholesterol and LDL levels was seen in the 3 groups. Neointima formation was significantly greater in the WD group.

Conclusions: ApoE-/- mice fed a WD diet develop HG, HI and an insulin resistant profile consistent with type 2 diabetes. FD fed mice maintain euglycemia but develop insulin resistance. Neointima growth at 28 days was significantly more robust in the WD group and intermediate in the FD group. These models may provide novel insights and an improved understanding of the atherogenic effects of high glucose, insulin resistance and hyperinsulinemia in the development of type 2 diabetes.

Diet CHO,mg/dL LDL,mg/dL Fasting Glucose,mg/dL Fasting Insulin,ng/ml No exposure 1306±100 113±100* 180±15 0.20±0.1 31000±2000 No exposure 1306±100 113±100* 180±15 0.20±0.1 31000±2000

1176-74
Increased Activity of Endogenous Endothelin-1 in Patients With Type 2 Diabetes Mellitus
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Background: Endothelial dysfunction in patients with diabetes may contribute to their risk of premature atherosclerosis. Endothelin (ET-1), a peptide released by endothelial cells, may be involved in this process by activating smooth muscle cell mitogenesis and leukocyte adhesion. We sought to assess the activity of endogenous ET-1 in a group of patients with non-insulin-dependent diabetes mellitus (NIDDM) by use of antagonists of ET-1 receptors.

Methods: Forearm blood flow (FBF) responses (strain gauge plethysmography) to intrabrachial infusion of a selective blocker of ET, receptors (BQ-123) and, on a different occasion, ET-1, were measured in 15 NIDDM patients and 12 healthy controls. On each occasion, 5 NIDDM patients received co-infusion of BQ-123 and BQ-788 (a selective blocker of ET, receptors).

Results: In normal subjects, BQ-123 did not significantly modify FBF from baseline (p>0.18) in NIDDM patients, in contrast, BQ-123 administration resulted in a significant vasodilator response (p<0.001). Inhibition of exogenous ET-1 resulted in lower vasoconstrictor responses in NIDDM patients than in controls (p=0.001), whereas vasoconstrictor responses to noradrenaline were similar in the 2 groups (p=0.78). In NIDDM patients, the vasodilator response to selective ET, blocker (BQ-123) was not significantly modified by co- infusion of BQ-123 and BQ-788.

Conclusions: The activity of endogenous ET-1 is enhanced in resistance vessels of NIDDM patients, and their sensitivity to exogenous ET-1 is blunted. Due to the atherogenic properties of ET-1, this abnormality may participate in the pathophysiology of the vascular disease in diabetic patients.

1176-75
Cigarette Smoke Creates an Unstable Athero-oclerotic Plaque Phenotype by Enhancing Vascular Oxidative Stress Factor Expression and Producing Collagen Loss in apo E−/− Mice
Takeo Anazawa, Juliana Yano, Xiaoying Zhao, Sanjay Kaul, Paul Dimayuga, Kuang-Yun Chyu, Bajan Cercari, Prediman K. Shah, Cedars-Sinai Medical Center, Los Angeles, California.

Background: The mechanism by which smoking increases risk for acute cardiovascular events is unclear. Using short-term cigarette smoke exposure, we have previously shown increased vascular oxidative stress and VEGF expression, in apoE-/* mice. The aim of this study was to determine the effect of cigarette smoke on plaque phenotype, vascular oxidative stress and VEGF expression in apoE−/− mice.

Method: After exposing mice to cigarette smoke (1 cigarette/day) for 28 weeks, aortic sinus plaque collagen and lipid content was measured. An additional group of mice were exposed to cigarette smoke for 8 weeks and their aortic exams were examined by Western blot for redox sensitive c-Jun and Ref-1. VEGF expression was studied by immunohistochemistry in aortic sinus plaques and Western blot of aortic extracts. Immunoreactivity is presented as percent stained area of plaque.

Results: Compared to no exposure (0%, n=6), redox sensitive c-Jun stain in apoE−/− mice was seen at 8 weeks of smoking, and this stain increased at 28 weeks. VEGF expression was increased in aortic sinus plaques at 8 weeks of smoking and increased further at 28 weeks. VEGF-positive cells were present throughout the plaque and were increased in the 28-week group. Additionally, plaque collagen content decreased in the 28 week group compared to the 8 week group. VEGF expression was increased in the 28 week group compared to the 8 week group.

Conclusions: Long-term exposure to cigarette smoke results in reduced plaque collagen content creating a more unstable plaque phenotype in apo E−/− mice. Evidence of enhanced redox sensitive gene expression (c-Jun and Ref-1) coupled with increased VEGF expression during short-term smoke exposure supports the hypothesis that increased vascular oxidative stress and angiogenic cytokines may mediate the effects of cigarette smoke on plaque phenotype.

1176-76
Selective Inducible Nitric Oxide Synthase Inhibitor Mercaptoethylguanidine Attenuates Neointimal Formation in Injured Mouse Exposed to Cigarette Smoke
Takeo Anazawa, Hongyan L. Xiaoanino Zhao, Juliana Yano, Kuang-Yun Chyu, Sanjay Kaul, Prediman K. Shah, Cedars-Sinai Medical Center, Los Angeles, California.

Background: Cigarette smoking and hypercholesteremias are synergistic risk factors of coronary heart disease. We have previously shown that INOS is associated with an increased arterial wall response to injury. We sought to determine the effects of smoking and hypercholesterolemia on the response to arterial injury in C57BI/6J mice and hypercholesterolemic ApoE−/−. The role of INOS in modulating the response was examined using selective INOS inhibitor mercaptoethylguanidine (MEG).

Methods: We used a mouse model of vascular injury induced by placing a periadventitial collar around the right carotid artery. Blood C57BI/6J mice were exposed to smoke of 1 cigarette/day, with (n=7) or without (n=4) MEG treatment. Sixteen ApoE−/− mice were exposed to cigarette smoke (n=12) or without (n=4) MEG treatment. Control C57BI/6J and ApoE−/− mice (n=11) were exposed to room air without MEG treatment. Expression of INOS was assessed by Western blot.

Results: Neointimal area (microm2) at 21 days after injury:

<table>
<thead>
<tr>
<th>Group</th>
<th>Control</th>
<th>Cigarette smoke</th>
<th>Cigarette smoke+MEG</th>
</tr>
</thead>
<tbody>
<tr>
<td>C57BI/6J</td>
<td>9.3±4 7.8</td>
<td>32.3±12.5</td>
<td>7.9±4.5</td>
</tr>
<tr>
<td>ApoE−/−</td>
<td>22.4±21.3</td>
<td>49±30.2</td>
<td>68.7±60.2</td>
</tr>
</tbody>
</table>

*p<0.05 vs. control; tp<0.05 vs. Cigarette smoke

INOS expression in MEG-treated C57BI/6J mice exposed to smoke was less compared to mice without MEG treatment.

Conclusion: Exposure to cigarette smoke increased neointimal thickening after arterial injury.