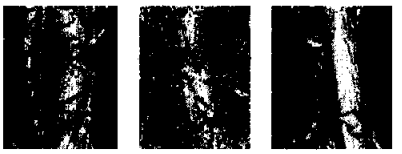
1176-72 Simvastatin Preserves the Structure of Coronary Adventitial Vasa Vasorum in Experimental Hypercholesterolemia Independent of Lipid Lowering

Joerg Herrmann, Stephanie H. Wilson, Erik L. Ritman, David R. Holmes, Jr., Lilach O. Lerman, Amir Lerman, *Division of Cardiovascular Diseases, Mayo Clinic Rochester, Rochester, Minnesota, Division of Anatomic Pathology, Mayo Clinic Rochester, Rochester, Minnesota.*

Background: Experimental hypercholesterolemia (HC) is associated with vasa vasorum (VV) neovascularization prior to lesion formation. Statins 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 (25-35 kg) were randomized to either normal diet (N, n=5) or high cholesterol diet without (HC, n=5) or with simvastatin supplementation (40-80 mg per day) (HC+S, n=4) for 12 weeks. The proximal LAD segment was scanned by 3D-microCT and VV density was determined in serial 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 (619±122 and 565±161 mg/dl, p<0.05). Vessel wall area (3.1±0.2 vs. 1.8±0.1 mm2) and VV density (4.7±0.3 vs. 2.7±0.2 n/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 n/mm2), despite similar increase in vessel wall area compared to N (2.5±0.1 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.



N HC HC + S

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

J William Phillips, Kurt G. Barringhaus, John S. Sanders, Ann C. Czarnik, Zandong Yang, Meng Chen, Jerry Nadler, Ian J. Sarembock, *University of Virginia, Charlottesville, Virginia.*

Hyperglycemia(HG) and hyperinsulinemia(HI) enhance the atherosclerotic complications of diabetes. Using a mouse injury model, we hypothesized that apolipoprotein E deficient(apoE-/-) mice fed a Western diet(WD) would develop HI with HG whereas mice on a Fructose diet(FD) would develop HI with euglycemia compared Chow diet(CD)and neointimal growth would be accelerated in WD fed mice.
Methods. Female ApoE-/- mice(n=10/group) were fed WD, FD, or CD for 1 week before wire 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 profiles and carotid arteries for histomorphometry were harvested.
Results. See Table. 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 develop HG, HI and an insulin release profile consistent with type 2 diabetes. FD fed mice maintain euglycemia but develop insulin resistance. Neointimal 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 atherosclerosis and the response to vascular injury in type 2 diabetes.

Diet	CHOL mg/dL	LDL mg/dL	Fasting Glucose mg/dL	Fasting Insulin ng/ml	Neointima μm^2
Western	1306±100	1133±100	160±15	0.20±0.1	31000μm^2±7000
Fructose	*720±100	*583±90	*113±30	0.15±0.05	*11000μm^2±2500
Chow	**454±80	**364±70	#107±18	#0.10±0.03	**5000μm^2±1000

*p≤0.05 WD VS. FD; **p≤0.05 FD VS.CD ; #p≤0.05 WD VS. CD

1176-74 Increased Activity of Endogenous Endothelin-1 in Patients With Type 2 Diabetes Mellitus

Umberto Campia, Carmine Cardillo, Melissa B. Bryant, Julio A. Panza, *Cardiology Branch - NHLBI - NIH, Bethesda, Maryland.*

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 intraarterial infusion of a selective blocker of ET_A receptors (BQ-123) and, on a different occasion, to ET-1, were measured in 15 NIDDM patients and 12 healthy controls. In addition, 5 NIDDM patients received co-infusion of BQ-123 and BQ-788 (a selective blocker of ET_A receptors).
Results: In normal subjects, BQ-123 did not significantly modify FBF from baseline (P=0.16); in NIDDM patients, in contrast, BQ-123 administration resulted in a significant vasodilator response (P<0.001). Infusion of exogenous ET-1 resulted in lower vasoconstrictor responses in NIDDM patients than in controls (P=0.001), whereas vasoconstrictor responses to norepinephrine were similar in the 2 groups (P=0.78). In NIDDM patients, the vasodilator response to selective ET_A blockade was not significantly modified by non-selective blockade of ET-1 receptors obtained by co-infusion of BQ-123 and BQ-788.
Conclusions:
The activity of endogenous ET-1 is enhanced in resistance vessels of NIDDM patients, whereas their sensitivity to exogenous ET-1 is blunted. Due to the atherogenetic properties of ET-1, this abnormality may participate in the pathophysiology of the vascular disease in diabetic patients.

1176-95 Cigarette Smoke Creates an Unstable Atherosclerotic Plaque Phenotype by Enhancing Vascular Oxidative Stress, Increasing Vascular Endothelial Growth Factor Expression and Producing Collagen Loss in apo E -/- Mice

Takeo Anazawa, Juliana Yano, Xioaning Zhao, Sanjay Kaul, Paul Dimayuga, Kuang-Yuh Chyu, Bojan Cercek, Prediman K. Shah, *Cedars-Sinai Medical Center, Los Angeles, California.*

Background: The mechanism by which smoking increases risk for acute cardiovascular events is unclear. By altering vascular oxidative stress, pro-inflammatory gene expression, and elaboration of angiogenic cytokines such as VEGF, smoking may enhance collagen degradation through MMP activation and change plaque composition. 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 26 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 extracts 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 positive stained area of plaque.
Results:

Long-term	Lipid area	Collagen area
No exposure	15.2±1.1%;n=3	55.3±7.5%;n=3
Cigarette smoke	17.2±7.6%;n=5	35.7±7.3%#; n=3

Short-term	c-Jun*	Ref-1*	VEGF*	% VEGF staint
No exposure	1.8±0.5 x 10^4	1.6±0.2 x 10^4	7.6±0.9 x 10^4	5.6±3.9% n=6
Cigarette smoke	3.2±0.4 x $10^{4\#}$	2.9±0.7 x $10^{4\#}$	10.2±0.9 x $10^{4\#}$	13.6±5.0%# n=8

*Relative densitometric units, n=4 each. †Percent stain of aortic sinus plaque.
#p<0.05 vs. No exposure.
Conclusion: 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 enhanced VEGF expression during short-term smoke exposure suggests that increased vascular oxidative stress and angiogenic cytokines may mediate the effects of cigarette smoke on plaque phenotype.

1176-96 Selective Inducible Nitric Oxide Synthase Inhibitor Mercaptoethylguanidine Attenuates Neointimal Formation in Injured Mice Exposed to Cigarette Smoke

Takeo Anazawa, Hongyan Li, Xioaning Zhao, Juliana Yano, Kuang-Yuh Chyu, Sanjay Kaul, Prediman K. Shah, Bojan Cercek, *Cedars-Sinai Medical Center, Los Angeles, California.*

Background: Cigarette smoking and hypercholesteremia 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 hypercholesteremia on the response to arterial injury in C57/Bl6J 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. Eleven C57Bl/6J mice were exposed to smoke of 1 cigarette/day, with (n=7) or without (n=5) MEG treatment. Sixteen ApoE -/- mice were exposed to cigarette smoke with (n=12) or without (n=4) MEG treatment. Control C57Bl/6J (n=6) and ApoE -/- (n=11) mice were exposed to room air without MEG treatment. Expression of iNOS was analyzed by western blot.
Results:

Neointimal area (microm sq.) 21 days after injury:

Group	Control	Cigarette smoke	Cigarette smoke+MEG
C57Bl/6J	9.3 +/- 7.6	23.3 +/- 12.9*	7.3 +/- 5.4†
ApoE-/-	22.4 +/- 21.3	49.0 +/- 30.2*	66.7 +/- 60.2

*p<0.05 vs. control; †p<0.05 vs. Cigarette smoke
iNOS expression in MEG-treated C57Bl/6J mice exposed to smoke was less compared to mice without MEG treatment.
Conclusion: Exposure to cigarette smoke increased neointimal thickening after arterial