		Death		MI	
OxLDL	<median (n=206)	8 (4%)		19 (9%)	
	>median (n=226)	9 (4%)	Ns	38 (17%)	P=0.02
OxLDL/HDL	<median (n=208)	9 (4%)		18 (9%)	
	>median (n=208)	6 (3%)	Ns	37 (18%)	P=0.006
LDL	<median (n=189)	5 (3%)		26 (14%)	
	>median (n=197)	9 (5%)	Ns	25 (13%)	Ns

Conclusions: The ratio of OxLDL to HDL seems to be an important predictor of subsequent MI in patients with UCAD.

POSTER SESSION

1064

Pathogenesis and Treatment of the Atherosclerotic Plaque

Monday, March 08, 2004, 9:00 a.m.-11:00 a.m.
Morial Convention Center, Hall G
Presentation Hour: 9:00 a.m.-10:00 a.m.

1064-178 A Novel Oral Neutrophil Elastase Inhibitor, ONO-6818, Suppressed Early Atherosclerotic Plaque Formation in Apolipoprotein E-Knockout Mice

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Objective: Proteolysis and elastolysis contribute to the development of atherosclerosis. Neutrophil elastase (NE) can degrade not only elastin but also collagens, fibronectin, and proteoglycans, and it is predominantly present in neutrophils. However, recent studies indicated the expression of NE in atheroma-related cells. Therefore, we hypothesized NE inhibition prevented atherogenesis, and examined whether ONO-6818, an orally active inhibitor of NE, limited atherosclerotic lesion in apolipoprotein E-knockout mice (ApoE-KO mice). Methods and Results: ApoE-KO mice (8 weeks of age) were fed a normal chow diet, and euthanized at the age of 12 weeks. They were divided into 2 groups; (1) control group (n=8, oral administration of saline) (2) treatment group (n=8, oral administration of 100mg/kg ONO-6818 for 4weeks). Plasma cholesterol levels were not significantly different between the two groups at the age of 12 weeks. Histological analysis of atherosclerotic lesions in the aortic root was performed using oil red O stained-sections. Plaque area was significantly reduced in ApoE-KO mice treated with ONO-6818 compared to the control group (9377 +/- 2078 µm2 versus 28464 +/- 7613 µm2, p=0.02). In immunohistochemical study, the percent area of macrophage accumulation to the total cross-sectional vessel wall area was smaller in ApoE-KO mice treated with ONO-6818 than the control group (1.0 +/- 0.2% versus 2.8 +/- 0.9%, p=0.07). The abdominal aortas of ApoE-KO mice were used for RT-PCR analysis of eNOS mRNA. ONO-6818 prevented downregulation of eNOS mRNA expression in ApoE-KO mice compared to the controls. Conclusions: NE inhibitor suppressed early atherosclerotic plaque formation and protected endothelial function in ApoE-KO mice. These results suggested that NE could be a new therapeutic target for atherogenesis.

1064-179 Mice Deficient in Leptin or the Leptin Receptor Exhibit Reduced Neointima Formation Following Vascular Injury

Yuechun Shen, Peter F. Bodary, Daniel T. Eitzman, University of Michigan, Ann Arbor, MI

Background: Elevated levels of plasma leptin have been identified as an independent risk factor for cardiovascular disease, although the mechanism by which leptin increases this risk is unknown. We hypothesize that leptin may contribute to vascular disease through effects on vascular remodeling.
Methods and results: A mouse model of femoral arterial wire injury was used that induces endothelial damage followed by intimal hyperplasia. We induced arterial injury in wild type (Lep+/+) (n=8), leptin deficient (Lep-/-) (n=13) and leptin receptor deficient (LepR-/-) (n=9) mice. Four weeks after injury, the intima/media ratios (I/Ms) of Lep-/- and LepR-/- mice were 0.80±0.14 and 0.55±0.20, which were significantly reduced compared to Lep+/+ mice (1.50±0.22) (p<0.01 and <0.02, respectively). To further establish that leptin was responsible for the altered neointima formation, leptin was replaced in Lep-/- mice by daily peritoneal injection beginning one day prior to injury until sacrifice. The I/Ms of Lep-/- mice (n=8) receiving leptin treatment were significantly higher (1.44±0.18) than Lep-/- mice described in the original cohort (p<0.02). Mice were also treated with an adenovirus, expressing murine leptin on the RSV promoter, one day following injury. The I/Ms of Lep-/- mice (n=6) receiving ad-leptin were 2.04±0.27, which were significantly higher compared to the I/Ms of the control Lep-/- mice (p<0.004). The I/Ms of LepR-/- mice (n=4) receiving ad-leptin were 0.50±0.19, which were not different from the I/Ms in the original group of LepR-/- mice.
Conclusion: These observations demonstrate that mice lacking leptin or the leptin receptor are protected from neointima formation following vascular injury and suggest that leptin may play a role in atherosclerosis by promoting lesion growth.

1064-180 Splenectomy and Adoptive Transfer of Splenocytes Reveal a Critical Role for Spleen in Mediating Athero-Protective Effects of Immunization With Apolipoprotein B-100-Related Peptide Sequence in Apo E (-/-) Mice

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Background: We have previously reported athero-protective effects of immunizing hypercholesterolemic apo E (-/-) mice with single or mixture of human apo B 100 related peptides. One such peptide is peptide 2 (ATRFKHLRKYTYNYEAESSS, 95% homology with murine apo B 100). In this study, we sought to determine the role of spleen in mediating athero-protective effects of peptide 2 immunization.
Methods and Results: Male apo E (-/-) mice were immunized with 33 mcg of peptide 2 at 6 weeks of age and boosted at 9 weeks with Alum as adjuvant. Control group received Alum only. At 12 weeks splenocytes were harvested, pooled and injected into non-immunized apo E (-/-) mice via tail vein. Both donor and recipient groups were fed high cholesterol diet until sacrifice at 25 weeks. Splenectomy abolished athero-protective effect of peptide 2 immunization; whereas adoptive transfer of splenocytes from peptide 2 immunized mice reduced atherosclerosis and cholesterol levels in recipient unimmunized mice. (Table)
Conclusion: Our data provide proof of principle that spleen plays an important role in mediating the atheroprotective effects of immunization with a single apo B 100 related peptide in apo E (-/-) mice.

	Cholesterol (mg/dl)	Aortic plaque (% area)	Aortic sinus plaque size (mm sq)	MOMA immunoreactivity (% plaque)
Alum donor, n=4	1285±114	5.6±5.3	0.55±0.05	4.3±4.6
P2 donor, n=9	910±459	3.0±1.7	0.45±0.18	4.2±3.5
Alum recipient, n=10	941±328	5.1±3.0	0.46±0.15	4.8±3.0
P2 recipient, n=9	560±239*	2.1±1.8*	0.33±0.13#	4.7±5.1

When compared to Alum recipient group "*" $p < 0.05$ and "#" p=0.07 by t-test

1064-181 Distinct Signaling Pathways Mediate Protease Activated Receptor-Dependent Endothelial Exocytosis

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Thrombin stimulation of the release von Willebrand Factor (vWF) and P-selectin from endothelial cells results in propagation of thrombotic events (vWF- mediated platelet adhesion and aggregation) and adhesion of neutrophils through P-selectin.The purpose of this study is to determine which Protease Activated Receptors are responsible for mediating thrombin-induced release of vWF and P-selectin from human umbilical vein endothelial cells (HUVEC). HUVEC were stimulated with thrombin, specific PAR-activating peptides (PAR-AP) or histamine and released vWF was measured in the media (ELISA), while P-selectin was measured on the cell membranes using a cell-based ELISA. PAR1-AP and PAR2-AP stimulation of vWF release was less effective when compared to either thrombin or histamine stimulation. However, PAR1-AP stimulation of P-selectin was as efficacious as histamine or thrombin stimulation. In contrast, PAR2-AP was significantly less efficacious in stimulating the release of P-selectin than vWF. PAR2-AP stimulation of mobilization of intracellular Ca^{2+} was nearly identical to PAR1-AP or thrombin stimulation of mobilization of intracellular Ca^{2+}. Calcium chelation with BAPTA nearly completely inhibited thrombin-mediated release of vWF while only inhibiting 50-60% of thrombin-mediated cell surface expression of P-selectin, suggesting an additional Ca^{2+}-independent pathway involved in release of P-selectin. Furthermore, pertussis toxin inhibited the release of vWF, while not effecting P-selectin release, implicating Gi/o in mediating the release of vWF, but not P-selectin. Immunostaining of P-selectin and vWF demonstrated co-localization of the two proteins in characteristic Weibel-Palade bodies. Differential signaling and co-localization of P-selectin with vWF suggest PARs stimulate the differential release of distinct fractions of Weibel-Palade bodies. Selective pharmacological targeting of specific PARs and G proteins provides a novel therapeutic strategy for altering the hemostatic and inflammatory balance of specific vascular beds.

1064-182 Gene Transfer of an ApoA-I Mimetic Peptide Reduces Atherosclerosis in Mice

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Background. Apolipoprotein A-I (ApoA-I) has been shown to be protective against vascular disease in animal models. The development of peptide-based mimetics of ApoA-1 may provide a particularly useful therapy for patients with atherosclerotic vascular disease. The goal of this study was to assess the effect of a novel ApoA-I peptide analogue (ESP 24218, Esperion Therapeutics) designed to activate LCAT and promote reverse lipid transport (RLT).
Methods and results. Nine week old male mice deficient in the LDL receptor were begun on a western chow diet to accelerate atherogenesis. At 14 weeks of age, mice were injected intravenously with saline (n=9) or a recombinant adenovirus expressing either wild-type ApoA-I (n=9), a 22 amino acid ApoA-I peptide analogue, ESP 24218, (n=10) or a 22 amino acid control peptide (n=10). At 18 weeks of age, mice were sacrificed and the atherosclerotic surface area was quantified in the aortic arch. The amount of atherosclerosis was significantly lower in mice expressing either wild-type ApoA-I