1007-100 Endothelial Dysfunction and Accumulation of Collagen are Associated With Restenosis and Constrictive Remodeling After Experimental Angioplasty

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Constrictive remodeling is related to restenosis after balloon angioplasty but its mechanisms remain unclear. Endothelial dysfunction and accumulation of extracellular matrix are known to occur after angioplasty. The aim of this study was to evaluate endothelial function and extracellular matrix in restenosis and arterial remodeling after experimental angioplasty.

Atherosclerosis was induced in femoral arteries of 15 New Zealand white rabbits by air-desiccation and high cholesterol diet. Four weeks later, angioplasty was performed. Histomorphometry and *in vitro* assessment of endothelial function were performed 4 weeks after angioplasty.

Restenosis correlated with constrictive remodeling ($r = 0.60$, $p = 0.01$) and with impaired relaxation via acetylcholine (Ach 10^{-6} M, $r = 0.61$, $p = 0.01$). Restenosis correlated with collagen accumulation ($r = 0.69$, $p = 0.004$) and inversely correlated with elastin content ($r = -0.53$, $p = 0.03$). Relaxation with Ach was significantly decreased in arteries with constrictive remodeling versus enlarged arteries (3.7 ± 7.9% vs 35.5 ± 15.0%, $p = 0.04$). Collagen content was significantly higher in arteries with constrictive remodeling than in enlarged arteries (34.5 ± 4.5% vs 18.2 ± 4.7%, $p = 0.03$). There was a trend for a decreased elastin content in arteries with constrictive remodeling versus enlarged arteries (18.9 ± 2.3% vs 24.9 ± 2.6%, $p = 0.10$).

Endothelial dysfunction and extracellular changes independently influenced both remodeling and restenosis.

Endothelial dysfunction and collagen accumulation might represent new targets to prevent restenosis.

1007-101 Delivery of the Gene Encoding Vascular Endothelial Growth Factor Alters Vascular Remodeling in an Atherosclerotic Model

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Vascular endothelial growth factor (VEGF) is a selective mitogen which enhances endothelial cell regrowth following arterial injury. To assess the effects of local production of VEGF on atherosclerotic vessel histology following angioplasty, we employed a recombinant adenovirus encoding murine VEGF1 (AdCMV-VEGF1).

Methods: New Zealand White rabbits were fed a 2% cholesterol diet for six weeks after balloon denudation of both iliac arteries. Following angioplasty of stenotic segments, vessels were exposed for 15 minutes to AdCMV-VEGF1 using a Transport infusion catheter. Control arteries were similarly treated with AdCMV-βGal, which codes for the biologically inert product βgalactosidase, or with AdRR5, a vector with no inserted gene. Animals were sacrificed 4 weeks later, and pressure-perfused serial sections were van-Giesson elastin stained for analysis of vessel components. Data below represent measurements at the minimal luminal area site of each artery.

VEGF (n = 9)	Control (n = 10)	p	
Lumen area (mm^2)	0.57 ± 0.24	0.31 ± 0.16	< 0.05
Plaque area (mm^2)	4.35 ± 0.79	3.81 ± 0.59	0.10
Vessel area (mm^2)	4.92 ± 0.73	4.12 ± 0.63	< 0.05
Plaque/Vessel	0.88 ± 0.05	0.93 ± 0.03	< 0.05

Conclusion: Delivery of AdCMV-VEGF1 tends to reduce plaque formation and significantly enhances remodeling, as evidenced by the larger vessel size, following angioplasty in this model.

1007-102 Differential Inhibition of Neointimal Thickening After Balloon Injury in the Rabbit Aorta by Glycosaminoglycans

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Background: Glycosoaminoglycans (GAGs) inhibit proliferation of cultured smooth muscle cells and some heparin preparations have been shown to attenuate restenosis, experimentally. In this study, we characterized the efficacy of GAGs with differing anticoagulant/antiproliferative profiles on neointimal thickening after arterial injury.

Method: Aortae of New Zealand White rabbits were injured with an overinflated 4F balloon catheter and harvested 2 weeks later. The animals received twice daily subcutaneous injections of either saline (control), 1.0 mg/kg heparan sulfate fraction (HS, CL-03405), 1.5 mg/kg dermatan sulfate-derived galnacuronans (DS, CL-03135) or 0.5 mg/kg Ardeparin (LMWH, Centaxann™) started 4 hours before injury. Verhoff's van Geisson stained aortic sections were digitally analyzed and the ratios of intimal to medial (I:M) area ratio calculated.

Results: The I:M area ratio after injury in the control group (n = 4) was 0.32 ± 0.07. Administration of either HS or LMWH, both of which are antithrombin III-dependent anticoagulants, significantly attenuated neointimal thickening, I:M ratios = 0.20 ± 0.06 (H, n = 5); and 0.14 ± 0.06 (LMWH; n = 5) ($p < 0.02$ for both vs control). The heparin cofactor II-dependent thrombin inhibitor DS was also effective, 0.21 ± 0.04* (DS; n = 5) ($p < 0.02$ vs control).

Conclusion: Each of the GAGs tested was effective in attenuating neointimal thickening after balloon injury, despite differences in their mechanism of anticoagulant action. HS which has been shown to be more effective than the LMWH tested in this study in inhibiting cultured smooth muscle cell proliferation (Karnovsky MJ, et al., 1995) was not more effective *in vivo*. The efficacy of GAGs in attenuating neointimal thickening after balloon injury appears to reflect both the anticoagulant and antiproliferative profile.

1007-103 Interplay Between Activation of the Endothelin System and Recovery of Endothelial NOS Following Arterial Balloon Injury

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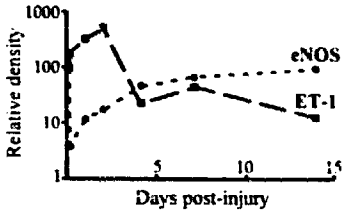
Background: Endothelin-1 (ET-1) and nitric oxide (NO) are potent vasoactive factors known to play a role in vascular remodelling.

Methods: This study assessed the temporal expression of endothelial NO-synthase (eNOS), ET-1 and ETA and ETB receptor mRNAs in the rat carotid artery after balloon injury using competitive reverse transcription and polymerase chain reaction and ribonuclease protection assay.

Results: ET-1 expression increased sharply following arterial injury, peaking (5.1-fold) at day 2. This was associated with a dramatic increase in ETB (63-fold) and ETA (158-fold) expression, peaking at day 1 and 2, respectively. The expression of eNOS was not detectable immediately after balloon injury, consistent with complete endothelial denudation, but reappeared after day 2, and increased progressively to preinjury levels by day 14. The recovery of eNOS expression mirrored the return of ET-1 and ET receptors to baseline levels.



Conclusions: These results confirm profound upregulation of the ET system in this model of arterial injury, and supports a critical role for re-endothelialization in the normalization of ET-1 and ET receptor expression during the recovery phase.

Moreover, endothelial NOS may be directly involved in restoring stability in the vessel wall possibly by reversing the synthetic phenotype of neointimal cells through ET blockade. [Supported by the HSFC]

1007-104 Balloon Injury of the Rabbit Aorta Induces Accumulation of Vitronectin and Vitronectin Receptor ($\alpha v\beta 3$), and Hypercholesterolemia Augments this Effect

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Background: Vitronectin (VN) is a multifunctional extracellular matrix and plasma protein that stimulates smooth muscle cell (SMC) migration and prolongs the activity of thrombin, effects that could promote neointimal hyperplasia. The VN receptor ($\alpha v\beta 3$) also mediates SMC migration and is a target of anti-restenosis therapy.

Method: In order to characterize the expression of VN and $\alpha v\beta 3$ after balloon injury, we developed a rabbit model using denudation of the abdominal aorta, with sacrifice at 1 day, 3 days, 1 week, 2 weeks, and 6 weeks after injury. We further studied 3 groups of rabbits at each time point. Group I was fed a normal diet (mean cholesterol at time of injury: 29 ± 3 and at sacrifice: 46 ± 18). Group II was fed a high cholesterol diet after injury (29 ± 3, 667 ± 137), and Group III was fed a high cholesterol diet for 6 weeks

prior to and after injury (*540 ± 311, 1658 ± 493). The aorta was fixed and immunohistochemistry was performed for VN and $\alpha v\beta 3$.

Results: In Group I, VN was detected as early as 1 day, with peak levels at 3 days and lower levels at 6 weeks. VN was primarily localized along the lumen at the earlier time points (suggesting deposition) and in the neointima and media at the later time points (suggesting synthesis). The time course and distribution of $\alpha v\beta 3$ was nearly identical to VN. In Groups II and III, the accumulation of VN and $\alpha v\beta 3$ was augmented and especially in the neointima.

Conclusion: We conclude that VN and $\alpha v\beta 3$ are detected early after balloon injury, consistent with roles in intimal hyperplasia. Notably, hypercholesterolemia markedly accentuated the accumulation of VN and $\alpha v\beta 3$ after balloon injury, suggesting a novel mechanism by which hypercholesterolemia may contribute to the progression of atherosclerosis and restenosis.

SUNDAY POSTER

1007-105 Human MCP-1 Is Upregulated In Restenotic Lesions When Compared With de Novo Lesions, In Vivo and In Vitro Study

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We reported previously the presence of monocyte chemoattractant protein-1 (MCP-1) gene in human coronary arteries. To determine whether MCP-1 is involved in the restenosis we examined MCP-1 expression by immunocytochemistry in atherectomy specimens and in smooth muscle cell (SMC) cultures derived from de novo (N) and restenotic (R) coronary atheromas. Tissue was obtained from 37 patients (25 w/de novo and 12 w/restenotic lesions). MCP-1 mRNA levels were analyzed by Northern Blots before (OD units) and after challenge with TNFα, PDGF, IGF-1 and Angiotensin II (AII) from 30 min. to 96 hours (%change over unstimulated control). MCP-1 was also measured in conditioned media by RIA.

Results: MCP-1 levels were higher in restenosis than id De novo lesions *atheromas ($p < 0.05$). This observation was verified by mRNA MCP-1 levels* in SMC cultures: 136.5 ± 38.6 ODU vs. 279.6 ± 67.3 ODU ($p < 0.002$), respectively. After the challenge MCP-1 mRNA levels in N increased as follows: TNFα by 226.4 ± 32.6% PDGF by 182.6 ± 25.2%, IGF-1 by 176.2 ± 21.3% AII by 186.5 ± 23.2% (all $p < 0.001$). In R MCP-1 levels did not changed after the challenge: 23.2 ± 23.1%, 13.4 ± 12.9%, 16.4 ± 21.4%, and 8.6 ± 12.6%, respectively (all NS). Analysis of MCP-1 in the conditioned medium revealed: N SMCc 5.5 ± 2.1 ng/10^3 cells, R lesions 22.3 ± 5.2 ng ($p < 0.01$). After the challenge: N SMCc TNFα 18.5 ± 6.4 ng/10^3 cells, PDGF 16.6 ± 5.4 ng/10^3 cells, IGF-1 14.9 ± 5.4 ng/10^3 cells and AII 12.5 ± 3.3 ng/10^3 cells (all $p < 0.001$). In R lesions: 26.5 ± 3.2 ng/10^3 cells 24.6 ± 4.1 ng/10^3 cells, 19.5 ± 3.2 ng/10^3 cells and 21.2 ± 4.2 ng/10^3 cells, respectively (NS).

Conclusions: Both, in vivo and in vitro study suggests that MCP-1 is significantly greater in restenotic than in de novo atheromas. In addition, MCP-1 gene in de novo lesions can be upregulated with PDGF, TNFα, IGF-1 and Angiotensin II. MCP-1 appears to be maximally upregulated in restenotic lesions. An important role for the MCP-1 protein in the restenosis process is implied.

1007-106 Apoptosis and Cell Proliferation Following Porcine Angioplasty

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Background: Angioplasty initiates a number of responses in the vessel wall, including cellular proliferation, which contribute to neointima formation and restenosis. Cellular homeostasis within a tissue depends on the balance between cell proliferation and apoptosis. The profiles of apoptosis and proliferation were therefore examined in a porcine PTCA injury model over a 28 day time period.

Methods: 42 arteries from 21 pigs, harvested at the site of maximal injury at 1, 6, 18 hours and 3, 7, 14 and 28 days post-PTCA (n = 3 animals per timepoint), using the uninjured arteries as controls, were examined. Apoptosis was demonstrated by TUNEL, TEM and DNA fragmentation, and cells traversing the cell-cycle were identified by immunostaining for PCNA.

Results: Apoptosis was not detected in control vessels nor at 28 days post-PTCA. Apoptotic cells were identified at early timepoints with a peak at 6 h (5.1 ± 0.26%; compared to uninjured artery $p < 0.001$) and confirmed by characteristic DNA ladders and TEM findings. In comparison, PCNA staining peaked at 3 days post PTCA (7.16 ± 0.29 %, compared to uninjured artery $p < 0.005$). The profiles of apoptosis and cell proliferation post-PTCA differed between traumatized and non-traumatized regions of the arterial wall. TEM and immunostaining with cell-type specific markers revealed that the apoptotic cells included VSMCs, inflammatory cells and adventitial fibroblasts.

Conclusions: The profiles of apoptosis and proliferation following PTCA are regional and cell-specific and attempts to modulate either of these events for therapeutic benefit requires recognition of these differences.

1008 Risk Factors and Markers of Coronary Artery Disease

Sunday, March 29, 1998, 5:00 p.m.–7:00 p.m.
Georgia World Congress Center, West Exhibit Hall Level
Presentation Hour: 5:00 p.m.–7:00 p.m.

1008-1 Elevated Serum Homocysteine Is Associated With Extent of Coronary Artery Atherosclerosis at Autopsy

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Background: Elevation of serum homocysteine has been associated with an increased risk of venous thrombosis, and, more recently, acute coronary events.

Methods: Hearts from 67 sudden non-coronary deaths and 152 cases of sudden coronary death (168 men and 61 women, mean age 50 ± 11 years), were studied by perfusion fixation and histologic examination of coronary arteries. Homocysteine levels were performed on postmortem sera.

Results: Coronary thrombi were noted in 97/152 cases of sudden coronary death. By univariate analysis, there was no correlation between coronary death and serum homocysteine levels (13.0 μM/L ± 9.8 for controls, 13.1 ± 8.3 μM/L for sudden coronary death), or between cases with coronary thrombi and those without (12.3 ± 7.5 μM/L for cases with thrombi, 13.6 ± 9.5 μM/L for cases without thrombi). The mean % maximal luminal narrowing of cases with homocysteine >20 μM/L was 78.6 ± 21% vs. 65.6% ± 31 for those with homocysteine ≤20 μM/L ($p = 0.03$). However, by multivariate analysis, only elevated cholesterol ($p = 0.04$), diabetes ($p = 0.05$), and cigarette smoking ($p = 0.06$) showed an association with maximal coronary narrowing, *independent of age, sex, and race.*

Conclusion: Homocysteine may be associated with severity of coronary atherosclerosis, but not coronary thrombosis or sudden death.

1008-2 von Willebrand Factor-dependent Shear-Induced Platelet Aggregation in Acute Myocardial Infarction

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Background: Recent *in vitro* studies suggested the crucial role of von Willebrand factor (vWF) and its interaction to platelet glycoproteins on platelet thrombus formation under the effect of blood flow. We have tested the effect of acute myocardial infarction plasma on vWF-dependent shear-induced platelet aggregation.

Methods: vWF-dependent shear-induced aggregation of platelets obtained from normal donors in the presence of plasma from either normal donors or fifteen cases of acute myocardial infarction was measured by an modified cone-plate viscometer. Antigen levels and ristocetin cofactor activities of vWF as well as plasma indicators of coagulation and fibrinolysis including thrombin antithrombin III complex (TAT) and plasmin anti-plasmin complex (PIC) were measured in all enrolled plasma.

Results: vWF-dependent shear-induced aggregation of platelets was enhanced by the addition of plasma from acute myocardial infarction from 32.3 ± 10.5% (mean ± SD) to 49.5 ± 19.8% ($p < 0.01$). Moreover, acute myocardial infarction plasma reduced the threshold level of shear stress necessary to cause vWF-dependent platelet aggregation from 90 dynes/cm^2 to 72 dynes/cm^2. Fibrinogen-dependent aggregation occurring under low shear stress (12 dynes/cm^2) was not influenced at all by the addition of myocardial infarction plasma. Enhanced vWF-dependent platelet aggregation may be explained by the increase levels of vWF antigen and ristocetin cofactor activities as well as relative increase in larger multimers in acute myocardial infarction.

Conclusion: vWF-dependent shear-induced platelet aggregation was enhanced in patients with acute myocardial infarction. This mechanism may be relevant to the onset and recurrence of myocardial infarction.

1008-3 Phenotype Characterization of Circulating Lymphocytes in Ischemic Heart Disease

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Background: Lymphocytes (LYMPHO) play an important role in the formation and evolution of coronary atherosclerotic plaques. The purpose of this study was to assess LYMPHO receptors expression in patients (pts) with various clinical manifestations of ischemic heart disease as well as to measure the