embryonic kidney epithelial cells which are competent to replicate these adenoviral vectors (293 cells). The vector incorporating the SMA promoter demonstrated substantial selectivity for vascular smooth muscle gene expression, with typical transductions carried out in parallel under identical conditions manifesting 90–95% lacZ-expressing BASMC, 0.3% lacZ-positive A549 cells, and 4% positive 293 cells. Conversely, parallel transductions with the vector employing the RSV promoter typically resulted in 95–99% lacZ-expressing 293 cells at vector concentrations yielding only 5–10% positive BASMC. These data support cell lineage-specificity of AvLacZ5 at the level of promoter function rather than due to intrinsic cellular differences in capacity for adenovirally-mediated transduction. However, it is notable that a limited subpopulation of 293 cells clearly are able to direct sufficient transcription from the SMA promoter sequences chosen to yield detectable lacZ expression; the molecular basis for this heterogeneity of expression remains to be determined. Adenoviral vectors utilizing these promoter sequences may render vascular-restricted gene transfer feasible when used in conjunction with mechanical devices providing a component of spatial localization.

1012-102 Alteration of Rabbit Carotid Artery Vasomotor Function by Gene Transfer with a Replication Deficient Adenovirus

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Gene transfer technologies offer great potential both to investigate and alter the course of vessel wall pathophysiology. Replication-deficient adenovirus vectors appear particularly useful for the transfection of blood vessels, because, of their ability to accommodate large cDNA inserts and to rapidly and efficiently infect mammalian cells. However, the potential effects of transfection with a replication-deficient adenovirus on vasomotor function have not been previously described. We performed gene transfer experiments with a replication-deficient adenovirus which contained a cytomegalovirus promoter and a nuclear-localizing variant of the bacterial β-galactosidase gene. Excised carotid artery segments from 6 rabbits were divided into multiple segments and infected in pairs, for 30 minutes with four different viral titers (0, 2.5×10^9, 5×10^9, 1×10^{10} pfu/ml) at 37°C in serum free media. These segments were tested for β-galactosidase staining and vasomotor function 2 days later. Isometric tension studies were performed to examine the response of the vessel segments to the contractile agonist, norepinephrine (NE). The results are shown below as mean ± standard error, $-\log_{10}[EC_{50}]$ with * $p \leq 0.04$ between the lowest and the highest group.

	Viral Titer (pfu/ml)			
	0	2.5×10^9	5×10^9	1×10^{10}
NE Dose	5.98 ± 0.09	5.77 ± 11	5.68 ± 0.14	5.52 ± 0.13*

*$p \leq 0.04$

In cell culture, rabbit vascular smooth muscle cells were infected in a similar manner. β-Galactosidase staining was present at all 3 viral concentrations but when a viral concentration of 1×10^{10} pfu/ml was used there were areas of cellular necrosis not seen at the lower viral concentrations.

Conclusion: Replication-deficient adenovirus vectors can rapidly and efficiently infect rabbit carotid vessels. However at doses often employed for gene transfer experiments *in-vivo*, there is a dose dependent alteration of vessel vasomotor function which may be mediated by a cytotoxic affect of the viral vector. This effect needs to be taken into account in studies involving adenoviral gene transfer.

1012-103 Restoration of Endothelial Function in Hypercholesterolemic Rabbit by Intermittent Administration of Vascular Endothelial Growth Factor

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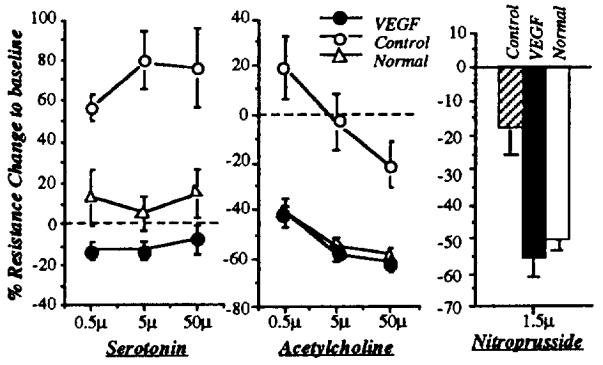
Endothelial dysfunction in hypercholesterolemia causes abnormal arterial vasoreactivity and precedes the onset of atherosclerosis. Vascular endothelial growth factor (VEGF) is an endothelial cell (EC) specific mitogen that has been shown to stimulate EC proliferation in vitro and in vivo. We investigated the hypothesis that VEGF could also modulate EC dysfunction and thereby improve endothelium-dependent vasoreactivity, impaired due to hypercholesterolemia. Hypercholesterolemic rabbits fed a 1% cholesterol diet for 6 weeks were treated with intravenous administration of 300 μg VEGF (VEGF group; n = 8) or 0.9% saline solution (control group; n = 9) twice a week for 3 weeks, during which time both groups continued to receive cholesterol diet. All rabbits were evaluated by in vivo vasomotion study; 8 normal (non-cholesterol and non-treated) rabbits were evaluated as well. The vasoreactive response to acetylcholine, serotonin and nitroprusside were calculated from flow velocity measured by Doppler wire, vessel diameter obtained from angiograms, and intra-arterial blood pressure recorded at the proximal external iliac artery. The resistance response to endothelium-dependent and -independent agonists recovered in VEGF group, as illustrated below. Furthermore, average intimal thickness of external iliac artery and lower abdominal aorta in VEGF group was significantly less than control group (VEGF = 0.010 ± 0.008 vs control = 0.106 ± 0.036, $p < 0.05$). *Conclusion:* Intermittent systemic administration of VEGF improves endothelial dysfunction and attenuates intimal thickness in hypercholesterolemic rabbit.



1012-104 Monoclonal Antibody to Tissue Factor Inhibits Intravascular Thrombosis without Impairing Extravascular Hemostasis

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Background: Recent studies suggest that the release of tissue factor (TF) and its interaction with factor VII at the site of vascular injury may be the activator of the coagulation cascade, which along with platelet aggregation leads to occlusive intravascular thrombus formation. We tested this hypothesis in a rabbit carotid artery model of thrombosis.

Methods: Carotid artery was instrumented with Doppler flow probe and a needle electrode. Partially occlusive thrombus was formed by applying 150 μA of current which damages the endothelium of the carotid artery and promotes thrombus formation. After development of 50% occlusion of the artery by thrombus, the current was stopped and a murine monoclonal antibody against rabbit TF (AP-1) (0.1 mg/kg intraarterially) or vehicle (control) was administered. The changes in carotid blood flow were continuously monitored by the Doppler flow probe. Bleeding was assessed by weighing the amount of blood absorbed in a preweighed sponge, placed in a cut wound that was 5 cm long and 0.5 cm deep.

Results: The control rabbits (n = 12) occluded their arteries in 46.2 ± 13.6 min after stopping the current by a fibrin-platelet thrombus. In contrast, AP-1 prevented carotid artery occlusion for >200 min (n = 6) ($p > 0.0001$). Lower doses of AP-1 were ineffective. The deep incisional bleeding times were not different between the control animals and the treated group. Similarly the platelet counts or *ex vivo* platelet aggregation response to collagen, ADP or arachidoic acid were not different between the control and the treated animals.

Conclusion: Data suggest that TF released at the site of vascular injury plays a role in intravascular coagulation and its activity can be selectively inhibited, without inducing an alteration in hemostatic parameters or platelet functions.

1012-105 Endothelin Immunoreactivity in Human Coronary Atherosclerosis

Amir Lerman, Kirk N. Garratt, David R. Holmes Jr., William D. Edwards, Linda J. McKinley, John C. Burnett Jr.. *Mayo Clinic, Rochester, MN*

Coronary atherosclerosis and restenosis are disease processes involving cell proliferation and activation of growth factors. Endothelin-1 (ET-1), a vasoconstrictor and mitogenic peptide, is produced from its precursor Big-ET. Coronary plasma ET concentrations are elevated in coronary atherosclerosis and following coronary interventions. The current study was designed to test the hypothesis that ET is produced and present in human coronary atherosclerotic and restenotic lesions and localized to both endothelial and nonendothelial cells. Twenty coronary lesions were obtained utilizing directional atherectomy. Immunohistochemistry for endothelins was performed with rabbit polyclonal ET-1 and Big-ET antiserum without cross reactivity be-

tween Big-ET and ET-1 antisera. In addition, staining for specific cell types, including macrophages (KP-1), endothelial cells (Factor VIII), and myointimal cells (actin), was performed. Eight primary native lesions, six restenotic lesions, and six vein graft (VG) lesions were studied. In all lesion types, intracellular Big-ET and ET-1 were present and in the extracellular matrix and colocalized to area with endothelial cells, macrophages, fibroblasts, and myointimal cells. ET-1 and Big-ET were colocalized to area with myointimal cells only in the primary native lesions. This study demonstrates the presence of Big-ET and ET-1 in coronary and VG atherosclerotic and restenotic lesions. This study suggests that ET is produced locally in the atherosclerotic and restenotic lesions by endothelial and nonendothelial cells. ET may play a role in the changes in tissue architecture and pathogenesis of coronary and VG atherosclerosis and restenosis.

1012-106 The Genotype of the ACE Gene and Collateral Formation

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The DD genotype of the angiotensin converting enzyme (ACE) gene has been reported to be a risk factor of myocardial infarction (MI), ischemic and idiopathic dilated cardiomyopathy, and left ventricular hypertrophy. The DD genotype of the ACE gene is associated with higher plasma ACE level, and thus may be associated with higher angiotensin II production in peripheral tissues. Because intimate involvement of angiotensin II in ventricular remodeling after MI has been reported, the genotype of the ACE could influence ventricular function after MI. The study population consisted of 66 subjects of MI who were performed twice coronary angiography (CAG) and left ventriculography(LVG). The first LVG was performed at 3.0 ± 2.9 months, and the second was performed at 10.2 ± 9.9 months from the onset of MI.

	ID + DD (n = 41)	II (n = 25)	P
EF 1st	0.568 ± 0.153	0.590 ± 0.116	0.547
EF 2nd	0.543 ± 0.133	0.623 ± 0.113	0.015
Collateral(+/−)	6/35	9/16	0.045

EF 2nd in subjects with either ID or DD genotype was significantly lower than in subjects with II genotype. Interestingly, presence of collateral circulation to infarct-related artery at the first CAG was more frequently observed in subjects with II genotype. To confirm the latter, we analyzed all subjects with ischemic heart disease who were performed CAG in our department in 1993.

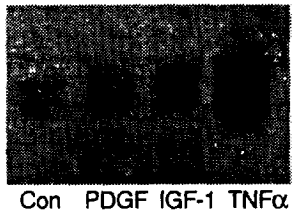
	ID + DD (n = 91)	II (n = 67)	P
Female/Male	19/72	15/52	0.820
AP/OMI	21/70	23/44	0.119
RCA + Cx/LAD	37/54	34/33	0.208
TIMI(0-2/3)	51/40	38/29	0.932
Collateral(+/−)	28/63	36/31	0.003

Thus, presence of collateral circuletion was more frequent in subjects with II genotype. This study suggests that the ACE genotype may influence left ventricular function after MI through affecting collateral formation.

1012-107 Monocyte Chemotactic Protein-1 Expression in Smooth Muscle Cell Cultures Derived from Human Coronary Arteries

M. Marius Rozek, R. Stefan Kiesz. *UTHSC at San Antonio, TX*

Peripheral blood monocytes and monocyte-derived macrophages are believed to play a pivotal role in the development of early atherosclerotic lesions. Excessive smooth muscle cell (SMC) proliferation leads to coronary restenosis after interventional procedures. We postulate that monocyte chemotactic protein-1 (MCP-1), by attracting monocytes to the site of vascular injury may indirectly augment this process. We also hypothesize that MCP-1 expression by human SMC may be mediated by cytokines and growth factors. Accordingly, we studied SMC cultures derived from tissue obtained by directional coronary atherectomy, evaluating MCP-1 expression by Northern blot analysis. Cells were grow in M-199 medium to over 95% confluence, for the last 24 hrs in serumless medium. MCP-1 expression was assessed in unstimulated (control) cells, and after 6 hrs stimulation with platelet derived growth factor (PDGF), insulin-like growth factor-1 (IGF-1) and tumor necrosis factor alpha (TNF_α). Autoradiographs were developed, scanned and digitized using NIH 1.53b10 software. Our findings suggest that unstimulated human coronary SMC derived cultures express MCP-1 and furthermore this expression is markedly increased after stimulation with cytokines (by 21.4% after stimulation with PDGF, 30.3% after IGF-1 and 119.1% after TNF_α).



Conclusions: SMC cultures derived from human coronary samples obtained by DCA express MCP-1 during their quiescent phase, and this expression is markedly increased by the cytokines PDGF, IGF-1 and TNF_α. Thus by attracting monocytes to the area of vascular injury, MCP-1 may be intimately involved in coronary restenosis, playing an important role in the pathophysiology of this process.

1013 Peripheral Vascular Disease — Thrombosis

Wednesday, March 22, 1995, Noon–2:00 p.m.
Ernest N. Morial Convention Center, Hall E
Presentation Hour: 1:00 p.m.–2:00 p.m.

1013-68 Postoperative and Late Prognosis for Diabetics Undergoing Vascular Surgery: Combining Clinical Evaluation and Dipyridamole-Thallium Imaging Improves Risk Stratification

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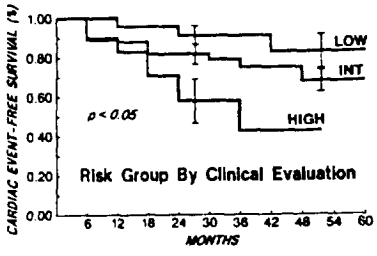
Prior studies suggest that pts with diabetes mellitus (DM) have a poor prognosis after vascular surgery and do not benefit from preoperative risk stratification by clinical evaluation or dipyridamole-thallium (DT) imaging because of a high prevalence of underlying coronary artery disease. To determine the postop and late prognosis for diabetics undergoing vascular surgery and the utility of clinical evaluation and DT for stratifying risk; we studied 122 pts with DM who underwent clinical evaluation and DT prior to vascular surgery. Based on the number of clinical markers present (using a previously described clinical risk index: prior MI, angina, Q wave on ECG, age > 70 yrs, CHF); pts were classified into low (0), intermediate (1–2), and high risk (>2 markers) for postop and late cardiac events. DT was assessed for reversible (REV) and fixed defects. There were 17 (14%) pts with postop events (4 cardiac deaths, 13 non-fatal MIs), and 23 (22%) with late events (8 cardiac deaths, 15 non-fatal MIs) on follow up which was possible in 98% of pts and was to 50 ± 5 months (75th quintile). On multivariate analysis, the only predictor for postop event was REV ($p < 0.01$) in this inherently high risk cohort. Successful risk stratification for late events was achieved by clinical index. DT was not of prognostic value for late event.



Conclusions: (1) Although diabetics are at high risk for events, dipyridamole-thallium imaging provides additional useful perioperative risk stratification. (2) The absence of clinical markers identifies a group with significantly better late cardiac event-free survival.

1013-69 Dipyridamole Rb-82 Positron Emission Tomography Has Limitations for Predicting Cardiac Events Peri- and Post-Operatively after Vascular Surgery

Kesavan Shan, Thomas Marwick, Raymundo Go, William MacIntyre, Donald Underwood, James Thomas. *Cleveland Clinic Foundation, Cleveland, OH*

Thallium imaging has been used to risk-stratify pts undergoing vascular surgery (VS), though the efficacy of this approach is debated. Positron emission tomography (PET) has the benefit of permitting a true resting scan, mea-