

	VLA-4 (n = 16)	Placebo (n = 15)	P
Lumen (mm ²)	0.18	0.23	0.75
Media (mm ²)	0.21	0.21	0.52
Intima (mm ²)	0.29	0.38	0.30
% Intima	0.59	0.61	0.60

Conclusion: Despite its ability to limit neointimal proliferation in transplant arteriopathy, VLA-4 blockade at the current dose has no significant effect on limiting neointimal proliferation following arterial injury in the rabbit atherosclerotic model.

1039-94 Effect of Latent Cytomegalovirus Infection of Rats on the Neointimal Response to Vascular Balloon Injury

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We recently found that prior cytomegalovirus (CMV) infection is a risk factor for restenosis after angioplasty, and acute infection of rats with rat CMV (RCMV), 1 day after carotid injury, increases neointimal accumulation. We hypothesized that vascular injury reactivates latent CMV; the reactivated virus then exacerbates the neointimal response to injury. Thus, to more critically test the CMV/restenosis hypothesis, we measured the neointimal response to carotid injury following latent RCMV infection. Sixty newborn male Sprague-Dawley rats (3 wks old) were randomized into two groups and received an *ip* injection of either 10⁶ active RCMV viral particles/ml or saline. Carotid balloon injury was performed 3 mos after infection, when no signs of infection were evident. Rats were sacrificed 6 wks later. Vessels were perfusion-fixed, cut into 3-5 segments, paraffin embedded, and stained by the Movat pentachrome method. The section with the greatest luminal narrowing was planimetricized and normalized by expressing the results as the neointima to media (N/M) ratio. We found that the N/M ratio of rats latently infected with CMV was 41% greater than uninfected controls (mean \pm SD: 1.40 \pm 0.48 vs 0.99 \pm 0.45, respectively; $P = 0.003$). The results were similar when analyzed as % stenosis (44 \pm 13% vs 32 \pm 14%, respectively; $P = 0.003$). We conclude that CMV has the genetic program that can cause excessive neointimal accumulation in response to vascular injury. The results are also compatible with our hypothesis that arterial injury reactivates latent virus, an event which then leads to cellular processes predisposing to restenosis.

1039-95 Restenosis is Highly Related to Collagen Content and Fiber Organization in the Atherosclerotic Rabbit Model

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Prior studies have shown that angioplasty stimulates an increase in both synthesis and degradation of collagen in the atherosclerotic vessel, however, differences in collagen content and metabolism between restenotic (R) and nonrestenotic (N) vessels have not been examined. Accordingly, collagen content in 11 R and N vessels was measured four weeks after angioplasty in an atherosclerotic rabbit model both biochemically by hydroxyproline (OH-pro) quantitation and histologically by a digital subtraction method using circularly polarized images of 20 picosirius red stained sections. Qualitative and quantitative histologic analysis of collagen fiber organization (CFO) was also performed. Collagenase and gelatinase activities were measured in the same R and N vessels using a radiolabeled substrate assay. Collagen content was found to be lower in R vs N vessels.

Collagen Content	R	N	p	n
Biochemically (μ g OH-pro/mg tissue)	127.0 \pm 32.6	212.6 \pm 84.3	< 0.05	11
Histologically (% intimal + medial area)	67.3 \pm 7.9	76.3 \pm 11.8	= 0.05	20

CFO in R vessels was less coherent with loose, more radially aligned fibers vs compacted, more circumferentially aligned fibers in N vessels. In N vessels, 80% of collagen fibers were < 20 $^\circ$ from tangential at the point of measurement vs 54% in R vessels ($p = 0.011$; $n = 50$ fibers). There was a correlation between lumen area and percent collagen content ($p = 0.0071$) and an inverse correlation between collagen content and gelatinase activity ($p = 0.02$). Thus, restenosis is highly related to collagen content and CFO which may be due to increased gelatinase activity.

1039-96 Endoluminal Versus Periadventitial Delivery of Antifibronectin Antibodies. Implications for Local Arterial Wall Delivery

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Periadventitial route has been suggested for drug delivery to the arterial wall. Its ability to achieve effective delivery of antibodies (Ab) to the media and intima is unknown. Total fibronectin (FNt) is expressed in rat arterial adventitia and intima. We compared endoluminal vs periadventitial delivery of rabbit polyclonal (IgG) FNt Ab, diluted at 1/20 either in PBS or in pluronic F127 (25% w/v). In 21 Sprague-Dawley rats, the left common carotid artery was exposed. In 8, the pluronic solution containing the Ab was applied onto the arterial adventitia and gelled instantly. In 13 other rats, endoluminal delivery was performed via a dwell method for 30 min. One day later, arterial cryosections were incubated with fluorescent (Texas red) Ab against rabbit to localise the FNt Ab. Controls included arteries either untreated or receiving non specific Ab. Periadventitial delivery via the pluronic gel resulted in labeling of the outer adventitia, but not of the media. The pattern was unmodified by periadventitial polyethylene wrapping or even adventitial stripping prior to delivery. Conversely, endoluminal delivery resulted in consistent labeling of the whole media, up to and including the adventitia. Kinetic studies (5, 15 min, 1, 3, 4 h) demonstrated progressive labeling of the media from the inner to its outer layers within 4 h. **Conclusion:** endoluminal delivery is the route of choice for Ab delivery to the arterial wall, achieving effective intimal, medial and adventitial delivery within 24 h. Conversely, periadventitial delivering of Ab do not allow medial penetration, whereas the endoluminal delivery appear to be the route of choice for specific Ab delivery to the media.

1039-97 Upregulated Arterial Expression of the Estrogen Receptor After Balloon Injury: Role in TGF- β Mediated Repair?

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Estrogens act through a specific receptor (ER), and are known to increase TGF- β expression. In human atherosclerosis and restenosis we previously documented the expression of β ig-h3, a TGF- β inducible gene that may serve as a biological marker of TGF- β activity. **Purpose:** Determine the arterial expression patterns of the ER and β ig-h3 after coronary artery angioplasty. Porcine coronary arteries were harvested 3, 7, 14 and 28 days after angioplasty [balloon:artery = 1.5:1.0; single injury (SI): $n = 6$; double injury (DI): $n = 6$]. Six control arteries were also studied. Proliferation, as assessed by incorporation of BrDU, was most pronounced (1-4% of cells) in all arterial layers on day 3 after SI and DI, and primarily involved adventitial and intimal smooth muscle cells (SMCs) and endothelial cells (ECs) of adventitial neovessels. By day 28 after DI adventitial SMCs appeared to migrate inward. Using two independent antibodies, ERs were immunolocalized to the nuclei of a few adventitial cells in normal arteries. In contrast, adventitial > medial > intimal SMCs over-expressed ERs with both cytoplasmic and nuclear distribution patterns early > late after SI and DI. Compared to normal arteries, β ig-h3 mRNA and protein were also over-expressed in adventitial SMC and EC early > late after SI and DI. **Conclusions:** Early after injury arterial expression of the ER is upregulated and is associated with the expression of a TGF- β inducible gene. The significance of ER expression in the artery wall is currently being studied, as it may enhance TGF- β expression and therefore, the role of this growth factor in vascular repair.

1039-98 Local Infusion of Vascular Endothelial Growth Factor Following Coronary Angioplasty in Swine

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The major limitation of PTCA is restenosis due to neointimal proliferation and vascular remodeling which is believed to be the result of endothelial denudation and medial injury occurring at the time of angioplasty. To investigate the hypothesis that accelerated reendothelialization would limit the proliferative response to vascular injury, we initiated experiments in pigs to locally infuse the endothelial-specific mitogen, Vascular Endothelial Growth Factor (V), following PTCA. LAD coronary arteries in 28 domestic pigs (weight 38.7 \pm 5.6 kg) were subjected to PTCA (balloon/artery ratio 1.3:1) followed by infusion of saline in 15 animals or 0.5 mg of V in 13 animals for 30 min via a local drug delivery catheter (DispatchTM). All animals receiving V experienced significant systemic effects. The systolic blood pressure decreased by 33.7 \pm 8.0 mm Hg within 5 min of initiating the V infusion requiring the use of pressors vs 6.8 \pm 4.9 mm Hg for the controls ($p < 0.0001$). The P_{O_2} decreased by 165.6 \pm 74.7 and 49.2 \pm 44.7 mm Hg for the V and control groups respectively ($p < 0.002$). These systemic effects resolved within minutes of stopping the

infusion. At 1 month the injured LAD segments were perfusion-fixed, excised and underwent quantitative histomorphometry. The area of neointimal growth was 0.428 ± 0.375 and 0.827 ± 0.388 mm² for the V and control groups respectively ($p < 0.03$). In conclusion: 1) V infusion was associated with a significant reduction in neointimal proliferation at 1 month following PTCA. 2) "Local" V infusion led to marked but reversible hypotension and hypoxemia possibly secondary to systemic vasodilation and increased vascular permeability.

1039-99 Extracellular Matrix Remodeling in Restenotic Human Coronary Atherosclerotic plaque of Balloon Angioplasty

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Although smooth muscle cell (SMC) proliferation is known to be an important factor for the development of restenosis after angioplasty, the role of extracellular matrix synthesis in restenosis has received little attention. The purpose of this study was to determine the sequence of changes in collagen, elastin, and proteoglycan synthesis and content after angioplasty-related vascular injury and to relate these changes to growth of the intimal lesions and extent of cell proliferation. We analyzed 164 restenotic coronary lesions obtained by coronary atherectomy histologically (HE, pentachrome, azan, and alcian blue stains) and immunohistologically (α -SM actin, CD-68 and PCNA labeling for cell proliferation). The restenosis population was divided into 4 groups of patients allocated to be recatheterized 1 to 2, 3 to 6, 7 to 9 and 10 to 12 months after successful angioplasty.

	Time course:			
	1-2 mo (n = 17)	3-6 mo (n = 82)	7-9 mo (n = 54)	10-12 mo (n = 11)
Shape of SMC	large	stellate	stellate	atrophic
PCNA to SMC	+	-	-	-
Proteoglycans	+	++	+	-
Macrophages	+	+	++	+
Collagen	-	-	+	++
Neointimaneovascularization	edema	healing	healed	

Neointimal stellate SMCs had no evidence of PCNA staining, while relatively large and round SMCs 1 to 2 months after angioplasty were strongly positive to PCNA staining. There was little proteoglycan accumulation at 1 to 2 months, but it dramatically increased from 3 to 6 months after angioplasty and then gradually decreased. These results suggest that extracellular matrix formation plays an important role in the development of restenosis lesion. Therapies aimed at controlling this process are worthy of further investigation to reduce restenosis.

1039-100 Natural Antioxidants Improve Regenerated Endothelium Dysfunction Following Balloon-Induced Vascular Injury

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Peroxidation of circulating and membrane lipids have been implicated in intimal hyperplasia and endothelial dysfunction following direct arterial trauma. The aim of this study was to study the effects of megadose of natural antioxidant vitamin E (VE) combined to vitamin C (VC) on the regenerated endothelium dysfunction. A 1st group of rats (n = 10) was pretreated with VE (500 IU/kg/day) and VC (1200 mg/kg/day) for 4 weeks before undergoing aortic (thoracic) endothelial denudation with a Fogarty catheter. Rats were then fed the same vitamin supplemented diet for 2 months. A 2nd group (n = 10), was similarly denuded and treated with soya oil (SO), VE vehicle, for the same period; a 3rd group (n = 10) was denuded only (DN); and a 4th group was maintained on a regular diet (CL) without denudation. Endothelial-dependent relaxation was assessed in organ chambers with acetylcholine (ACH) and endothelium-independent relaxation and smooth muscle contractility were evaluated with sodium nitroprusside (SNP) and phenylephrine (PE) respectively. Endothelial regeneration was evaluated with Evans blue staining. Vascular relaxation (SNP) and contractility (PE) were not affected either by the regenerative process or the vitamin supplementation. However, endothelial-dependent relaxation to ACH was significantly improved in animals treated with VE [(maximal relaxation %), CL: 82 ± 5 , VE: 82 ± 4 , SO: 61 ± 5 , DN: 47 ± 7], $p < 0.01$]. Morphometric studies with Evans blue staining showed over 95% regeneration of the endothelial surface of the denuded aortas of all groups. Plasmatic VE concentrations were significantly increased in VE-treated group (VE: 40.7 ± 2.5 vs SO: 21.9 ± 1.3

$\mu\text{M/L}$, $p < 0.01$). These results suggest that, in this model, dietary megadose of antioxidant vitamin initiated 4 weeks before denudation can improve the post-regenerative endothelial dysfunction without significantly affecting smooth muscle function.

1039-101 Expression of Collagen And Elastin After Coronary Injury

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The accumulation of collagen and elastin contributes to arterial remodeling. To examine temporal and spatial characteristics of type I collagen and elastin synthesis after coronary injury, a porcine model was used. Normal arteries (n = 6) demonstrated weak collagen and elastin expression. At 3 days after balloon injury (n = 3), procollagen $\alpha 1(I)$ transcripts and protein, not $\alpha 1(III)$, were markedly elevated in the adventitia. At 7-14 days after injury (n = 7), procollagen $\alpha(I)$ was augmented in adventitial and neointimal cells, but not in the media. At 30-90 days (n = 5), procollagen $\alpha(I)$ levels returned to baseline in the adventitia, but they remained elevated in neointima. The media exhibited low levels of procollagen $\alpha(I)$ at all times after injury. The above changes were paralleled by the accumulation of cross-linked type I collagen in the adventitia (onset at 7 days) followed by neointima (onset at 14 days). The expression and synthesis of elastin differed. At early stages after coronary injury, a low basal level of elastin was only present in the media. A marked increase in elastin was observed in the adventitia and neointima at 90 days, whereas no changes were noted in the media.

Conclusions: (1) adventitial and neointimal cells demonstrate concordant synthesis of extracellular matrix proteins; (2) collagen and elastin are involved in different stages of vascular remodeling after coronary injury; (3) the sequential increase in collagen and elastin expression in the adventitia and neointima provides additional evidence that neointimal cells are derived from adventitial fibroblasts.

1039-102 Adriamycin Induces Apoptosis and Antiproliferative Effects in Smooth Muscle Cells From Human Restenotic Lesions

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Increased proliferation and/or decreased programmed cell death (apoptosis) of smooth muscle cells (SMCs) are currently discussed as pathogenic mechanisms leading to intimal hyperplasia/restenosis. Therefore, we examined the effects of adriamycin (Adm) as anti-tumour agent on SMC proliferation and apoptosis. To this end, human SMCs were cultivated from restenotic tissue and subsequently taken for flow cytometry (FACSscan). SMCs expressed strong positive signals for PCNA and cyclin E, but negative signals for cyclin B and cyclin D1-3, indicative of S-phase growth events. *In vitro* proliferative activity of restenotic SMCs was quantitated by growth curves and expressed in population doubling rates (PDR). To detect SMC apoptosis, we performed FACSscan by use of propidium iodide as well as transmission electron microscopy (TEM). Results were as follows ($x \pm SD$; n = 5 experiments; * $p < 0.01$).

	Proliferation (PDR)	Apoptotic SMCs/total SMCs (%)
Control	0.44 ± 0.02	12 ± 5
Adm 10 μM	$0.17 \pm 0.05^*$	$48 \pm 13^*$

As shown in the Table, proliferation of restenotic SMCs indicated by high PDR was markedly suppressed by Adm. Likewise, the portion of apoptotic SMCs was found significantly increased by Adm, as seen by their reduced DNA content (FACSscan). Additional TEM analysis revealed extensive cell shrinkage, membrane budding and formation of apoptotic bodies in Adm-treated SMCs, thereby signalling characteristic features of apoptosis. In conclusion, our data show Adm to be an attractive candidate for local drug delivery due to its considerable growth-inhibitory and pro-apoptotic efficacy, in particular for restenotic SMCs.