Conclusions: This study suggests that mean plaque size stabilizes at 4 weeks after carotid injury with no further increase at later time points. Furthermore, there appears to be a time dependent decrease in activated macrophages in the neointima. Potential mechanisms for this observation are currently being investigated.

1200-143 Altered AP-1/Ref-1 Redox Pathway in iNOS Deficient Vascular Smooth Muscle Cells: A Novel Involvement of iNOS in Cellular Signaling

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Background: We previously showed injury-induced medial proliferation and neointimal formation in carotid arteries of inducible nitric oxide synthase knockout (iNOS KO) mice were significantly less compared to wild type (WT). iNOS is a source of reactive oxygen species, which could modulate cellular growth and redox signaling molecules such as the AP-1/Ref-1/thioredoxin pathway.
Hypothesis: SMC deficient in iNOS have altered redox sensitive AP-1/Ref-1 signaling pathway and reduced proliferative response to serum stimulation.
Methods: Confluent aortic SMC isolated from iNOS KO and WT mice were stimulated to proliferate with 20% serum media after 48 hours of quiescence. Cell cycling by FACS analysis and nuclear PCNA and thioredoxin by Western blot was characterized 24 hours after stimulation. The following assays were performed 30 minutes after stimulation: AP-1 DNA binding activity by gel-shift assay; cJun mRNA by semi-quantitative RT-PCR and cJun and Ref-1 expression by Western blotting.
Results: Cell cycle analysis showed significantly more iNOS KO cells remained in the G0/G1 phase and less in S phase after 24 hours of serum stimulation. cJun and Ref-1 expression and AP-1 activity were also less in iNOS KO SMC (Table). Thioredoxin expression was also less in iNOS KO cells.
Conclusion: Our data demonstrated reduced proliferative response and altered AP-1/Ref-1 signaling pathway in iNOS KO SMC, implying a novel signaling mechanism for iNOS involvement in modulating SMC proliferation.

Cell Type	S-phase	PCNA	AP-1 Gel-shift activity	c-jun mRNA	Nuclear c-Jun	Nuclear Ref-1 (x 10^3)
WT	26.9±18.8 %	8.8±2.7	2.0±0.7	1.79±0.11	6.1±3.8	6±2.5
iNOS KO	8.5±3.7%*	2.0±2.0*	1.0±0.2*	1.08±0.26*	0.7±0.5*	0.09±0.06*

PCNA, AP-1, nuclear c-Jun and c-jun mRNA are expressed as fold change relative to unstimulated cells; Ref-1 is expressed as relative densitometric units; *p<0.05. Each experiment was repeated at least 3 times

POSTER SESSION

1201 Gene Transfer to Vascular Tissues and Cell Therapies

Tuesday, April 01, 2003, 3:00 p.m.-5:00 p.m.
McCormick Place, Hall A
Presentation Hour: 4:00 p.m.-5:00 p.m.

1201-117 A Combination of Transcriptional Regulatory Elements Increases Transgene Expression 40-Fold in Porcine Coronary Arteries

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SMC are relatively refractory to adenovirus mediated gene transfer, due in part to poor transgene expression, even from powerful viral promoters. The murine cytomegalovirus promoter (MIEmCMV) significantly improves transgene expression in SMC compared to the widely used human CMV promoter (MIEhCMV). Inclusion of the Woodchuck Hepatitis Virus Post Transcriptional Regulatory Element (WPRE) and a fragment of the rabbit smooth muscle myosin heavy chain promoter (RE) further increases expression of *lacZ* ≈5 fold *in vitro*. We hypothesised that this combination would significantly increase transgene expression *in vivo*. **Methods:** Porcine coronary arteries randomly received 2x10^9iu of the following vectors in 300µl of vehicle (n=4/group) via an Infiltrator™:
1. Ad5-*lacZ*: MIEhCMV
2. RAd36: MIEmCMV
3. Ad5-PP-*lacZ*: MIEmCMV, WPRE
4. Ad5-PREP-*lacZ*: RE, MIEmCMV, WPRE.

After 72 hours β-galactosidase expression and activity was quantified. **Results:** Ad5-PREP-*lacZ* induced 40 times greater β-gal activity than Ad5-*lacZ* (p<0.05) and greater activity than the other MIEmCMV based vectors (both p<0.01). X-gal staining was 7-35 fold greater in Ad5-PREP-*lacZ* infected vessels (all p<0.001). **Conclusion:** Inclusion of WPRE and RE within the cassette of a recombinant adenovirus vector regulated by MIEmCMV substantially improves transgene expression in porcine coronary arteries. By maximizing expression or allowing use of lower virus doses this has important implications for successful gene therapy in the vasculature.

	Ad5-*lacZ*	RAd36	Ad5-PP-*lacZ*	Ad5-PREP-*lacZ*	p (ANOVA)
X-gal staining (µm^2)	1519 ±503	462 ±181	1915 ±507	16381 ±4483	<0.0001
β-gal activity (IU/µg protein/min)	0.05 ±0.02	0.11 ±0.06	0.07 ±0.02	2.05 ±1.46	<0.006

1201-118 Blockade of TGF-Beta by Catheter-Based Gene Transfer of a Soluble TGF-Beta Type II Receptor Inhibits Neointima in a Porcine Coronary Artery After Stenting

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Background: Enhanced extracellular matrix (ECM) accumulation is an important finding in porcine as well as in human coronary stent restenotic tissue, and TGF-β, implicated in ECM formation, is expressed abundantly in these tissue. We assessed the hypothesis that blockade of TGF-β by local delivery of an adenovirus expressing a soluble form of TGF-β type II receptor (AdTβ-ExR) inhibits stent-induced neointima in porcine coronary arteries.
Methods: Two remote coronary arterial segments (n=20) per each pig were randomized to receive 1x10^9 pfu of either AdTβ-ExR or adenovirus expressing β-galactosidase (AdLacZ) using an infiltrator. A stent (n=20) was deployed after gene transfer in each segment in 10 pigs. Localized expression of transgene was confirmed by both reverse transcription-PCR and immunohistochemistry. Computer-based morphometric assessment was performed in stented arteries at 4 weeks after gene transfer.
Results: There were significantly less ratio of intima area (IA)/media area (MA) and higher neointima cell density in stented arteries treated with AdTβ-ExR comparing with those with AdLacZ. Neither cell replication rate assessed by PCNA immunohistochemistry nor injury score was significantly different between two groups.
Conclusion: Blockade of TGF-β by local *in vivo* gene transfer of a soluble TGF-β receptor inhibits stent-induced neointima by inhibiting ECM accumulation in porcine coronary arteries, and may provide a therapeutic potential to inhibit restenosis after stenting.

Morphometric assessment of stented arteries after gene transfer

	IA/MA	MA	stent area	neointima cell density
AdTβ-ExR	0.84±0.44*	2.2±0.8 mm^2	5.4±0.6 mm^2	3663±597/mm^2*
AdLacZ	1.21±0.45	1.9±0.6 mm^2	5.6±0.7 mm^2	2800±190/mm^2

*p<0.05 vs AdLacZ

1201-119 Adenovirus Mediated Prostacyclin Synthase Gene Transfer Inhibits Neointimal Formation by Modulating Peroxisome Proliferator-Activated Receptors Expression

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Peroxisome proliferator-activated receptors (PPARs) are nuclear hormone receptors which regulate cell growth and differentiation by modulating gene transcription. Many data demonstrate that PPARα and γ are expressed in human atherosclerotic lesion and their ligands like fibrates and troglidazone reduce neointimal formation after angioplasty. However, little is known about the role of PPARα in regulatory mechanism of arterial remodeling. Prostacyclin (PGI_2) is a potent ligand of PPARα and we have shown that PGI_2 synthase (PCS) gene transfer accelerates reendothelialization through induction of cyclooxygenase-2 and vascular endothelial growth factor (VEGF) and prevents neointimal formation in balloon-injured arteries. To further explore pathophysiological relationship between PGI_2 overproduction and PPARα expression in balloon-injured arteries, we carried out chronospatial analysis of PPARs (α, γ and δ) and evaluated arterial morphometry after adenovirus-mediated PCS gene transfer(AdPCS). Imunohistochemical analysis revealed that 1)PPARα was positive in endothelium in balloon-injured groups, 2)all PPAR subtypes were expressed in neointimal area at day 7 and 14, while little was found in uninjured vessels, 3)In AdPCS group, PPARδ and γ distribution was limited to the neointima adjacent to endothelium, while, in control, diffuse expression was seen in whole neointima. The concentration of tissue 6-keto-Prostaglandin$F_{1\alpha}$, a metabolite of PGI_2, was significantly increased in AdPCS group (AdPCS vs control; 5.35+/-0.95 vs 2.29+/-0.54 ng/mg tissue, p=0.02). Morphometric analysis at day 14 revealed that AdPCS reduced intima/media ratio up to 40% (AdPCS vs control; 0.83+/-0.05 vs 1.32+/-0.13, p=0.01). In conclusion, PCS gene transfer could inhibit neointimal formation by suppressing smooth muscle cell proliferation and migration partly via PPARδ pathway as well as acceleration of reendothelialization via cyclooxygenase-2-VEGF pathway.

1201-120 Inhibition of Angiogenesis and Wound Healing by Adenovirus-Mediated Gene Transfer of a Soluble Form of Vascular Endothelial Growth Factor Receptor in Mice

Johannes Jacobi, Betty Y. Tam, Uma Sundram, Calvin J. Kuo, John P. Cooke, Stanford University, Stanford, CA

Background: Vascular endothelial growth factor (VEGF) is an important angiogenic growth factor. Since angiogenesis plays a major role in wound repair, we hypothesized that adenovirus-mediated gene transfer of a soluble form of VEGF receptor 2 (Flk-1) might attenuate wound healing in mice.