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.

Altered AP-1/Ref-1 Redox Pathway in INOS Deficient Vascular Smooth Muscle Cells: A Novel Invovlement of INOS in Cellular Signaling

Kuang-Yu Chyu, Xiaoming Zhao, Paul C. DiRosa, Bojan Corcos, Predmien K. Chugh, Cedars-Sinai Medical Center, Los Angeles, CA

Background: We previously showed injury-induced medial proliferation and neointimal formation in carotid arteries of normoxia norexoxo symmetrical knockout (eNOS 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 and Ref-1 signaling pathways.

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 thoredoxin by Western blot was characterized 24 hours after stimulation. The following assays were performed 30 minutes after stimulation: AP-1 UNE binding activity by gel-shift assay; cJun mRNA by semi-quantitative H1-PLH and c-Jun 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 G phase after 24 hours of serum stimulation. cJun and Ref-1 expression and AP-1 activity were also less in INOS KO SMC (Table). Thoredoxin 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.

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>S-phase</th>
<th>PCNA</th>
<th>c-Jun mRNA</th>
<th>c-Jun cjun</th>
<th>Nuclear Ref-1</th>
<th>Nuclear Ref-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT</td>
<td>26.9±16</td>
<td>8.8±2</td>
<td>2.0±0.7</td>
<td>1.79±0.1</td>
<td>6.1±3</td>
<td>6.2±2.5</td>
</tr>
<tr>
<td>INOS</td>
<td>8.5±3.7</td>
<td>2.0±1</td>
<td>0.2±1.2</td>
<td>1.0±0.26</td>
<td>2.7±0.9</td>
<td>0.09±0.06</td>
</tr>
<tr>
<td>KO</td>
<td>0±1.2</td>
<td>5±1.2</td>
<td>0±1.2</td>
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</table>

PCNA, c-Jun c-Jun and c-jun mRNA are expressed at different levels in unstimulated cells. c-Jun is expressed as a 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.
McConmick Place, Hall A
Presentation Hour: 4:00 p.m.-5:00 p.m.

A Combination of Transcriptional Regulatory Elements Increases Transgene Expression 40-Fold In Porcine Coronary Arteries

Clare E. Apley2, Paul A. Kingston, Anne David, Anthony M. Haegerty, University of Medicine, Manchester, United Kingdom

SMC are relatively refractory to adenovirus mediated gene transfer, due in part to poor gene expression, even from powerful viral promoters. The murine cytoptagaladivirus promoter (MIEhCMV) 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 thioredoxin by Western blot was characterized 24 hours after stimulation.

Results: There were significantly less ratio of intima areas (IA)/media area (MA) and media area (MA) and intima taken at 24 hours of serum stimulation. cJun and Ref-1 expression was also less in INOS KO SMC (Table). Thoredoxin 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.

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PCNA, c-Jun c-Jun and c-jun mRNA are expressed at different levels in unstimulated cells. c-Jun is expressed as a relative densitometric units; >p>0.05. Each experiment was repeated at least 3 times.

Poster Session

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

Ick-Moo Chung, Hijukku Ueno, Donghoon Choi, Yongsang Jiang, Ji-La Shin, Youngki N. Pak, Junwoon Kim, Chan Park, Ewha Women’s University, Seoul, South Korea, University of Occupational and Environmental Health, Kitakyusyu, Japan

Background: Enhanced extracellular matrix (ECM) accumulation is an important finding in porcine as well as in human coronary stent restenotic tissue, and TGFβ, 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 (AdTβR) inhibits stent-induced neointima in porcine coronary arteries.

Methods: Two remote coronary arterial segments (n=20) per each pig were randomized to receive 1x10⁹ pfu of either AdTβR or adenovirus expressing β-galactosidase (AdlacZ) using a Stentor. A stent (n=20) was deployed after gene transfer in each segment in 10 pigs. Localized expression of transgene was confirmed by reverse transcription-PCR and immunohistostaining. Computer-based morphometric assessment was performed in stented arteries at 4 weeks after gene transfer.

Results: There were significantly less ratio of intima areas (IA)/media area (MA) and media area (MA) and intima taken at 24 hours of serum stimulation. cJun and Ref-1 expression was also less in INOS KO SMC (Table). Thoredoxin 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.

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

Hajime Inai, Yasushi Numaguchi, Yasuhito Nishimoto, Hideo Matsui, Toyoko Murohara, Kenji Okumura, Nagoya University, Nagoya, Japan, Children's Hospital, Boston, MA

Peroxisome-proliferator-activated receptors (PPARs) are nuclear hormone receptors which regulate cell growth and differentiation by modulating gene transcription. Many data demonstrate that PPARα, PPARγ and PPARδ are expressed in human atherosclerotic lesion and their ligands like steroids and tigascogtid reduce neointima formation after angioplasty. However, little is known about the role of PPARs in regulatory mechanism of arterial remodeling. Prostacyclin (PGI₂) is a potent ligand of PPARα and we have shown that PGI₂ synthase (PGS) gene transfer can inhibit endothelial dysfunction induced through inhibition of cyclooxygenase-2 and vascular endothelial growth factor (VEGF) and prevents neointimal formation in balloon-injured arteries. To further explore pathophysiological relationship between PGI₂ production and PPARs expression in balloon-injured arteries, we carried out chronocasial analysis of expression of PPARα, PPARγ, and PPARδ and evaluated arteriome necrosis mediated vascular gene transfer (AdPCPG). Immunohistochromatic analysis revealed that PPARα was positive in endothelium in balloon-injured groups, while PPARδ and PPARγ were positive in intimal and medial layers, respectively. In balloon-injured groups, PPARα, PPARγ, and PPARδ expression was limited to the neointima adjacent to endothelium, while, in control, diffuse expression was seen in whole neointima. The concentration of tissue PGI₂ level, PPARα, PPARγ, and PPARδ expression was significantly increased in AdPCPG group (AdPCPG vs control; 5.35±0.05 vs 2.28±0.54 ng/ml, tissue, p<0.05). Morphometric analysis at day 14 revealed that PPARα was positive in intimal/media ratio in AdPCPG group (AdPCPG vs control; 6.12±2.6 vs 1.0±0.13, p<0.01). In conclusion, PPARα gene transfer could inhibit neointimal formation by suppressing smooth muscle cell proliferation and migration partly via PPARα 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

Jonathen Jacobs, Betty Y. Tam, Uma Sundram, Calvin J. Koo, 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 (Fn1) might attenuate wound healing in mice.