by ELISA. Abciximab had a dose-dependent inhibitory effect on both α and dense PGR that paralleled the inhibition of aggregation. Decreased PGR may enhance both the long and short term therapeutic benefits of abciximab by minimizing mural thrombus formation and decreasing the amount of mitogen release at the site of vascular damage.

Abciximab (μg/mL)	% Inhibition of aggregation†	% Inhibition of release		
		ATP*	β-TG†	PAI-1†
0.5	5 ± 5.3	14 ± 13.5	17 ± 5.1	6 ± 4.5
1.5	15 ± 7.7	24 ± 22.4	18 ± 11.0	25 ± 7.6
2.0	35 ± 3.7	59 ± 14.4	62 ± 5.9	58 ± 5.3
2.5	54 ± 6.8	64 ± 18.2	82 ± 5.3	76 ± 0.9
5.0	99 ± 1.4	72 ± 13.8	87 ± 2.5	82 ± 1.2
10.0	100 ± 0.8	81 ± 2.0	87 ± 3.3	81 ± 1.7

*TRAP (4 to 6 μM); †ADP (5 μM). Data is the ave ± 1 standard deviation

1010 Gene Transfer

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.

1010-6 Gene Transfer to the Pulmonary Vasculature Using Genetically Engineered Smooth Muscle Cells

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Background: To circumvent the problems of *in vivo* transfection and avoid the use of viral vectors or proteins, we sought to establish whether smooth muscle cells (SMC) transfected *ex vivo* could be delivered via the systemic venous circulation into the pulmonary bed, achieving local transgene expression in the lung.

Methods: Primary cultures of pulmonary artery SMC's from Fisher 344 rats were labeled with a fluorescent, membrane-impermeable dye (CMTMR), or transfected with the beta-galactosidase reporter gene under the control of the CMV enhancer/promoter (pCMV-β). Transfected or labeled SMC's (5×10^6 cells/animal) were delivered to syngeneic recipient rats by injection into the jugular vein, the animals were sacrificed at intervals 15 minutes to 2 weeks later, and the lungs excised and examined.

Results: At 15 minutes post transplantation, injected cells were detected mainly in the lumen of small pulmonary arteries and arterioles, often in groups of 3 or more cells. After 24 hours, labeled SMC's were found incorporated into the vascular wall of pulmonary arterioles, and transgene expression persisted in situ for 14 days, with no evidence of immune response and minimal attrition. Using simple geometric assumptions, it was calculated that approximately 45 ± 16% of the transfected cells reintroduced into the venous circulation could be identified in the lungs after 1 hour, 25 ± 9% at 24 hours, 13 ± 6% at 1 week and 16 ± 6% at 2 weeks (n = 6–8 for each group).

Conclusion: These results suggest that a cell-based strategy of *ex-vivo* transfection may provide an effective nonviral gene transfer approach for the treatment of pulmonary vascular diseases.

1010-7 Adenoviral Gene Transfer of Human Renal V_2 Vasopressin Receptors Improves Contractility in Cardiomyocytes

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Background: In heart failure, increased concentrations of arginine vasopressin (AVP) contribute to vasoconstriction and reduced cardiac contractility. These effects are mediated via the V1 subtype of vasopressin receptors (V1R) which stimulates intracellular IP3 accumulation. The V2 subtype (V2R) is not expressed in the myocardium physiologically. V2R expression is restricted to the kidney, where V2R activation stimulates adenylyl cyclase. Overexpression of V2R in the myocardium might allow to use AVP for an inotropic effect via a heterologous, cAMP-coupling receptor.

Methods: We constructed a replication-deficient adenovirus for the human V2 vasopressin receptor (AdV2R). Cardiomyoblasts were infected with 10–1000 pfu AdV2R/cell. Expression of the recombinant V2R was assessed by means of 3H-AVP binding in combination with specific V1/V2 antagonists. We detected a maximum V2R density of 792 ± 105 fmol/mg protein, whereas control virus-infected cells only showed V1R binding. Maximum V2R expression was reached 72 h after infection. Confocal immunofluorescence laser microscopy showed V2R expression in >90% of infected cells. AVP induced a 30-fold cAMP increase with an EC50 of 25 pM. Recombinant V2R-expressing, spontaneously beating neonatal cardiomyocytes showed a 40% increase in contractility after AVP stimulation at an EC50-value of 10–100 pM.

Conclusions: A functionally active adenovirus for the human V2 receptor was constructed. After infection of cardiomyocytes, AVP stimulation of V2R-expressing cells led to an increase of intracellular cAMP accumulation. The heterologous expression of the cAMP-forming receptor induced contractile responses to AVP in cardiomyocytes.

1010-8 High Frequency Ultrasound Enhances Transfection of Porcine Vascular Smooth Muscle Cells *in Vitro*

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Background: Progress in vascular gene therapy has been hindered by the limited efficiency and/or toxicity of current transfection techniques. Ultrasound is safe and has been shown to facilitate transfection of fibroblasts *in vitro*. We have investigated the effects of of high frequency ultrasound on transfection of porcine aortic vascular smooth muscle cells (VSMCs) *in vitro*.

Methods: Subconfluent VSMCs (passages 2–7) were transfected with naked or liposome (Promega Tfx-50)-complexed luciferase plasmid DNA (7.5 μg/ml) in medium containing 10% (v/v) porcine serum; continuous wave ultrasound at 0 (control), 1, 3 or 10 MHz was applied for 60 s; luciferase activity was assayed at 48 h and expressed as light units per μg total cell protein (LU/μg); cell counts were performed at 0, 3, 18 and 48 h.

Results: Exposure to ultrasound at 1 MHz was associated with a 7.5-fold increase in transfection with naked DNA (DNA alone 0.4 ± 0.2 LU/μg; with 1 MHz treatment 3.0 ± 2.0 LU/μg, p = 0.028) and a 3 fold enhancement with liposome-complexed plasmid (liposome/DNA 27.6 ± 6.9 LU/μg; with 1 MHz treatment 72.8 ± 17 LU/μg, p = 0.007). Treatment with 3 or 10 MHz had no effect on transfection efficiency. Treatment with 1 MHz resulted in a 41% reduction in cell number at the 48 h timepoint compared with control ($P < 0.05$).

Conclusion: High frequency ultrasound enhances transfection of VSMCs *in vitro* and may also retard VSMC proliferation, both desirable properties in the context of the prevention of restenosis.

1010-9 Direct DNA Injection of Vascular Endothelial Growth Factor Induces Angiogenesis in Non-Ischemic Myocardium

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Background: Previous work has demonstrated the ability of vascular endothelial growth factor (VEGF) to induce angiogenesis after direct injection of naked plasmid DNA in peripheral muscle. We determined if this technique could provide an effective method of in-vivo myocardial gene transfer.

Methods: Plasmid DNA for the 165 amino-acid isoform of VEGF under the control of the cytomegalovirus enhancer/promoter ($pVEGF_{165}$) was injected into the left ventricular free wall of Sprague-Dawley rats aged 10–12 weeks. Control animals were injected with either plasmid DNA coding for the reporter gene beta-galactosidase (pCMV-β) or with buffered saline. Animals were sacrificed at intervals from 48 hours to 2 weeks and the hearts examined.

Results: Significant beta-gal staining was detected 7 days after injection, with approximately 3–5% of the volume of the left ventricle expressing the transgene. The hearts injected with pCMV-β or buffered saline demonstrated a 3 fold increase in the number of vessels along the course of the needle tract and a 6 fold increase in the total vessel area as compared to non-injected myocardium. Hearts injected with $pVEGF_{165}$ demonstrated marked neovascularization with a 4 fold increase in the total number of vessels and a 15 fold increase in the total vessel area, as compared to the pCMV-β injected hearts. By 14 days after injection the number and total area of vessels had decreased to non-injected control levels in all animals.

Conclusion: These results indicate that direct myocardial injection of plasmid DNA is an effective method of *in-vivo* gene transfer, and transfection with VEGF induces significant but transient neovascularization of non-ischemic myocardium, greater than that seen with needle trauma alone.