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A Novel Surgical Approach Towards Survival After Gene Delivery in the Mouse

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With the advent of gene therapy there has been much interest towards gene delivery into the heart. Prior to human therapy, delivery modalities must be examined in animal models. The mouse is an excellent model to examine the molecular and genetic effects of gene expression, however it has not been practical to directly deliver gene products into the coronary arteries due to technical limitations. We describe a surgical technique that allows direct perfusion of the coronary arteries in a mouse with subsequent survival and expression or reporter genes. Mice were sedated and intubated with an angiocatheter into the trachea attached to a rodent ventilator. A median sternotomy was performed. The common carotid artery was ligated distally under a microscope. The ascending aorta was temporarily occluded with a vascular clamp. Access to the ascending aorta was obtained by injecting 250 µl of Adenovirus particles containing a CMV promoter expressing β-Galactosidase (LacZ)through a 30 gauge needle into the proximal common carotid artery. The needle was withdrawn and the proximal common carotid was ligated and transected between the two ligation points. The ascending aortic clamp was removed and the chest wall was sutured closed. Once the animal regained consciousness, the tracheal catheter was removed and the animals were sacrificed after 72 hours. Hearts are then frozen and sectioned and stained for LacZ. We have found approximately 5% of the myocardium stained for LacZ in a diffuse manner, confirming global delivery. *Conclusion*: We describe the first surgical approach of direct coronary infusion of gene products into the mouse heart permitting survival of the animal. We believe this model can be used to evaluate the efficacy and effects of gene therapy. Further work to optimize conditions and maximize gene delivery need to be pursued.

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Immunosuppressive Gene Delivery of Naked DNA into a Heterotopic Cardiac Allograft Model

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The only definitive treatment for end stage congestive heart failure is cardiac transplantation. Unfortunately, many patients succumb to the detrimental systemic effects of current immunosuppressive therapy. Organ specific gene therapy may curtail these systemic side effects. Local gene delivery into the myocardium has been validated in animal models using a variety of delivery techniques. We studied the effect of coronary perfusion of naked DNA with immunosuppressive cytokines on survival in a vascularized heterotopic cardiac allograft mouse model. We injected donor hearts with Lactated Ringers (control), a plasmid (MCVIZ) with the cardiac specific α-Myosin Heavy Chain Promoter (α-MHC) expressing Viral Interleukin 10 (vIL-10), Internal Ribosomal Entry Sequence (IRES) and the β-Galactosidase (LacZ) gene (10, 20 and 40 µgm), or a plasmid (MCIV) containing IRES and LacZ (20 µgm). *Results*: Control animals (n=4) survived a mean of 10.25 ± 1.49 days. MCVIZ treated animals at concentrations of 10, 20 and 40 µgm (n= 6,3,3) survived 24.33 ± 9.43 (p=.0957), 15.33 ± .88 (p=.0409), 16.33 ± 1.20 (p=.0409) days respectively. MCIZ animals at 20 µgm survived 15.00 ± 1.15 days (p=.0690). *Conclusion*: Naked plasmid DNA utilizing the cardiac promoter α-MHC expressing the immunosuppressive cytokine vIL-10 along with IRES and LacZ injected into donor hearts enhance survival in a mouse heterotopic cardiac allograft model. Interestingly, the expression plasmid MCIZ which does not express vIL-10 trended towards enhanced survival however this was not statistically significant. Immune modulation due to foreign nonspecific DNA or LacZ expression must be considered. Further analysis needs to be performed to understand this potential immunosuppressive effect.

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Increased Myocardial Osteopontin Expression Coincides with the Transition from Hypertrophy to Failure

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To identify genes differentially expressed during the transition from hypertrophy to failure, myocardial mRNA from spontaneously hypertensive rats with heart failure (SHR-F) was compared to age-matched SHR with compensated hypertrophy (SHR-NF) and normotensive WKY rats by differential display RT-PCR. SHR-F showed labored breathing, pleuropericardial effusions, atrial thrombi and RV hypertrophy. Characterization of a transcript differentially expressed in SHR-F yielded a cDNA with homology to the extracellular matrix protein osteopontin (OPN). Northern analysis showed low levels of OP mRNA in LV myocardium from WKY and SHR-NF, but a markedly increased (12±4.3-fold vs. SHR-NF; $p<0.05$; $n=5$) level in SHR-F. In myocardium from WKY and SHR-NF, in situ hybridization showed only scant OPN mRNA, primarily in arteriolar cells. In SHR-F, in situ hybridization revealed abundant expression of OPN mRNA primarily in non-myocytes in the interstitial and perivascular space. Treatment with captopril abolished the increase in LV OPN mRNA levels, suggesting a role for angiotensin II (AII). To confirm if AII can increase OPN expression, cardiac myocytes, microvascular endothelial cells and fibroblasts isolated from adult rat heart were treated with AII in vitro. This treatment increased OPN gene expression 4-5 fold in microvascular endothelial cells and fibroblasts but not in myocytes. Thus, OP expression is markedly increased in the heart coincident with the transition from hypertrophy to failure. The source of OPN in SHR-F is primarily non-myocytes, and AII seems to be involved in OPN gene expression. Given OPN's known biological activities including cell adhesion and regulation of inducible nitric oxide synthase gene expression, these data suggest that it plays a role in the pathophysiology of myocardial remodeling and failure.