996-11 Direct Gene Transfer and Expression with Arterial Iontophoretic Catheter Delivery

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Iontophoresis is a technique of molecular delivery which uses electric current to enhance movement of charged molecules into tissues. A porous balloon catheter was tested with a central silver chloride electrode capable of generating a potential gradient across the arterial wall using an adhesive patch placed on the skin to serve as the anode. We hypothesized that this catheter delivery system might effectively transfer negatively charged plasmid DNA into arterial cells *in vivo*.

Methods: To localize plasmid DNA arterial delivery, a 7 Fr iontophoretic porous balloon catheter was placed into porcine carotid arteries under flouroscopic guidance. 15 μg of ^{35}S-labeled plasmid DNA (1.4×10^6 cpm/μg) expressing the heat stable human alkaline phosphatase (hAP) gene with an RSV promoter was infused through the balloon at 6 atm pressure. A constant current density of 2.5 mA/cm^2 was maintained for 10 minutes. The ^{35}S-labeled plasmid DNA delivery was repeated on the contralateral carotid artery under identical conditions with the absence of electric current. 20 minutes after gene transfer, the arteries were fixed *in situ* and processed for autoradiography. To analyze gene transfer and expression, 8 porcine carotid arterial segments were subject to iontophoretic gene delivery for 10 minutes at 6 atm with a current density of 2.5 mA/cm^2 using the RSV hAP plasmid (n = 6) or control plasmid (n = 2). Animals were sacrificed 5 days after gene delivery and the transfected arteries analyzed by PCR and heat stable alkaline phosphatase histochemistry.

Results: Autoradiography of the arteries which underwent ^{35}S-labeled plasmid delivery revealed minimal radiolabel in the luminal cells of the control artery in which current was not delivered. In contrast, significant amounts of radiolabel were present in the media and adventitia of the artery subject to current delivery. PCR analysis of the arterial segments studied 5 days after delivery confirmed gene transfer in all hAP segments and was negative in control arteries. Staining for heat stable recombinant alkaline phosphatase activity demonstrated recombinant protein expression in 5% of medial cells and 10% of adventitial cells in arteries which underwent hAP gene transfer. Control arteries were negative for hAP staining.

Conclusions: Iontophoretic catheter gone delivery can be used to perform direct plasmid DNA delivery with expression of recombinant protein in medial and adventitial cells.

996-12 Overexpression of Sarcoplasmic Reticulum Calcium ATPase in Neonatal Cardiomyocytes Using a Recombinant Adenovirus

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Cardiac Sarcoplasmic Reticulum Calcium ATPase (SERCA2) is a membrane pump that plays a major role in regulating calcium levels in cardiac myocytes. Recent data have documented a decrease in SERCA2 levels in human heart failure. In order to further study the importance of SERCA2 levels in cardiac cellular physiology we constructed a recombinant replication deficient adenovirus encoding SERCA2 driven by an SV40 enhanced TK promoter (14CTK). After documenting the presence of the SERCA2 gene by southern analysis of viral DNA, neonatal rat cardiomyocytes were infected with either the SERCA2 encoding adenovirus or an adenovirus encoding beta-galactosidase. Cells were harvested for RNA preparation at 48 hours and for protein at 72 hours post infection. Northern blot analysis using a full length probe specific for rat SERCA2 revealed a several fold induction of SERCA2 mRNA levels compared to cells infected with the beta-galactosidase encoding virus. Western blot analysis with a polyclonal SERCA2 antibody revealed an approximate 2.5 fold induction of SERCA2 protein. These data demonstrate that SERCA2 can be overexpressed in cardiac myocytes using this adenoviral vector based approach. Functional studies of infected cardiomyocytes are currently underway.

996-13 Inflammation Following Adenovirus-mediated Direct Gene Transfer into Porcine Coronary Arteries and Strategies for Its Control

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The vascular response to any given combination of gene transfer vector and catheter delivery system is an important factor to consider in developing gene therapy strategies for preventing coronary restenosis. In order to study the vascular response to adenoviral (Ad5) vectors following transluminal delivery by perforated balloon catheter, we constructed a replication-deficient Ad5 expressing a nuclear-localized β-galactosidase and deployed it in porcine coronary arteries using Wolinsky Infusion Catheters (USCI, Billerica, MA). Viral infections were performed under fluoroscopy by infusing 4 ml (2×10^{10} pfu) of the virus into the coronary arteries of intubated swine at 8 atm of pressure. Three days later, the arteries were removed and fixed prior to a 6 hr incubation in X-gal. The nuclear localization of the indigo reaction product provided for unambiguous detection of Ad5-infected smooth muscle cells in the media; however, specific staining of cells in the near adventitia was also noted suggesting distribution of virus by the vasa vasorum. The endothelial denudation and medial disorganization observed in Ad5-infected arteries were similar to that found in balloon-injured and vehicle-infused control arteries. The most remarkable histologic finding was a pronounced cellular infiltrate in the adventitia and periadventitia of the Ad5-infected arteries that was absent in the vehicle-infused controls. Immunohistochemical staining of arterial cross-sections using a monoclonal antibody directed against the porcine lymphocyte homing receptor (CD44) identified the infiltrating cells as leukocytes. These results suggest that Ad5-mediated gene transfer to the coronary arteries of Hanford miniature swine elicits an inflammatory response mediated by cytotoxic T-cells, monocytes/macrophages, and/or natural killer cells. The natural response of the host to viral infection may therefore contribute to the precipitous decline in Ad5-mediated gene expression observed in this animal model and others. Potential strategies for controlling the inflammatory response to Ad5 vectors will be discussed, including immunosuppression of the host, modifications to the Ad5-genome, and the long-term possibility of using synthetic complexes to emulate vital gene transfer without eliciting inflammation.

996-14 Hypoxia-induced Alterations in Gene Expression in Cardiac and Endothelial Cells

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Altered gene expression patterns accompany episodes of hypoxia both with and without reoxygenation. We are using cultured cardiac myocyte and endothelial cell models to identify genes that are differentially regulated by oxygen levels. This study employs a PCR-based RNA fingerprinting technique, differential display, that allows the sampling of the effects of hypoxia on gene expression over multiple time points with and without reoxygenation. Differential display was performed using total RNA isolated from cardiac AT-1 cells or endothelial cells. Cells were exposed to two main experimental protocols: anoxia for six hours with or without a four hour reoxygenation, or 24 hours of hypoxia (1% oxygen). Oxygen tension in the culture media was monitored using a silver-platinum electrode. Total RNA was isolated and used as the template for reverse transcriptase reactions and subsequent differential display PCR reactions. Three candidate differentially expressed genes have been identified in the initial set of experiments in AT-1 cells. These results suggest that this fingerprinting methodology will enable the identification of oxygen-regulated genes and will increase our understanding of the molecular basis of cellular responses to hypoxia.

996-15 Adenovirus Mediated Inducible Heat Shock Protein 70 Gene Transfer Protects Against Simulated Ischemia in a Muscle Derived Cell Line

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The inducible heat shock protein 70 (HSP70i) is a stress related factor that has been shown to have a protective effect against ischemia. In order to study further this protective effect we have constructed a recombinant replication deficient adenovirus that constitutively expresses HSP70i at high levels under the direction of the viral CMV promoter. Expression was verified in neonatal cardiomyocytes in cell culture infected at a multiplicity of infection of 2:1. The majority of cells stained strongly positive after 24 hours with immunohistochemistry using a monoclonal antibody against HSP70i. Neonatal cardiomyocytes infected similarly with a recombinant adenovirus encoding beta-galactosidase did not stain with the HSP70i antibody. C2C12 cells, a muscle cell line, were infected with either the HSP70i adenovirus or beta-galactosidase encoding adenovirus as above. After 24 hours the cells were incubated with rhodamine, a fluorescent marker retained by viable cells, and were then subjected to simulated ischemia (slightly hypotonic Hanks buffer in a GasPak hypoxic jar) for 6 hours. After a 1 hour non-hypoxic recovery period in isosmotic media the cells were examined with a fluorescent microscope. There was a readily apparent increase in survival of the HSP70i adenovirus infected cells relative to the beta-galactosidase infected cells based on rhodamine mediated fluorescence. These studies demonstrate the feasibility of using adenoviral vectors to effect expression of HSP70i in cardiac myocytes and also demonstrate the efficacy of this approach for providing protection against simulated ischemia.